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PROCEEDINGS
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SOME OBSERVATIONS ON THE ERROR OF BIO-ASSAY

By Edwin B. Wilson

Office of Naval Research, Boston

Communicated December 31, 1951

Recently Berkson[1] has given data on eight different methods of determining L.D.50. In ten samples six of the eight methods were applied; in five samples all eight.[2] This sort of statistical study of what actually is found when different methods are applied to a variety of similar samples is of real scientific importance in showing how far theory is borne out in practice and how far different methods differ in actual results. I take no exception to any of Berkson's comments; I merely wish to add some.

Omitting the two methods which were applied to only five samples, the data are:

TABLE 1

L.D.50 by Six Different Methods in Ten Samples

1	0.686	0.995	1.23	0.982	0.939	0.907	0.092	0.983	0.903	0.199
2	0.687	0.998	1.22	0.982	0.939	0.928	0.093	0.985	0.913	0.199
3	0.695	0.970	1.26	0.965	0.925	0.915	0.120	0.990	0.913	0.194
4	0.680	1.025	1.23	0.945	0.936	0.920	0.100	0.982	0.908	0.198
5	0.653	1.049	1.27	0.974	0.922	0.908	0.104	0.991	0.910	0.203
8[a]	0.689	1.014	1.24	0.994	0.976	0.947	0.105	0.019	0.921	0.203

[a] I keep Berkson's numbering. The methods are: 1, probit, maximum likelihood; 2, logit, minimum X^2; 3, probit line fitted graphically; 4, logit line fitted graphically; 5, Reed-Muench method; 8, straight line fitted by eye.

When one desires to compare different methods, one of the simplest things he can do is to serialize the results in each sample, assigning 1 to that method which gives the least value, 2 to the next, and so on. If this be done for the ten samples of table 1, one perceives at once that method 8 is rated 4, 5 or 6 in all ten. The probability, on the natural heads-tails null hypothesis, that any one method will be rated in either half of the possible ratings, high or low, on ten samples is $2/1024 = 0.002$,

and that some one of six shall be found so rated is 0.012. The inference is that method 8 runs systematically high relative to the other five.[3]

I shall therefore discard it as making the 6 by 10 table statistically inhomogeneous and confine further remarks to the other five methods. If these be serialized, it is seen that methods 1 and 4 are running low; methods 2 and 5 are high; and 3 is near normal. If we sum deviations of the serial numbers from their mean of 3 over the ten samples, we get for the five methods the respective values: −5, 4.5, 2, −5.5, 4. The distribution has a bit of a *U*-type look, but its variance is about right.

Whether any further detail is advantageous over that obtained through serialization may be doubted. The standard method of variance analysis seems to be inapplicable owing to the notable difference in the variances of the five entries in the ten columns. Even the number of significant figures in the variation within the column varies so that the different columns should probably have different weights in determining the relative departures of the different entries.

Notwithstanding, it is tempting to use the method of converting the five entries in each column to standard measure. Result:[4]

TABLE 2

DEVIATIONS IN STANDARD MEASURE TO TENTHS

											MEAN
1	4	− 5	− 6	9	9	−11	−10	− 9	−15	1	−3.3
2	5	− 3	−11	9	9	16	− 9	− 3	6	1	2.0
3	10	−14	9	− 3	−10	− 1	17	11	15	−16	1.8
4	0	7	− 6	−18	5	6	− 2	−11	− 5	− 2	−2.6
5	−19	15	14	3	−14	−10	2	13	0	15	1.9

One may compare the means in the last column with the results obtained by serialization; the correlation is high. The five means are closer to zero than expected; the standard deviations in rows 1, 2, 4 are smaller and in rows 3, 5 larger than expected; but these results are not significant.

[1] Berkson, J., *Biometrics*, **6**, 432–434 (1950).

[2] In these PROCEEDINGS in volume 29 at various times I gave with Jane Worcester a method of determining L.D.50 and its standard deviation of sampling obtained by applying the method of maximum likelihood operating upon a logistic. The method and the tables prepared for ready use assumed a specific experimental set-up suited to routine testing in bacteriological laboratories. The page references to these articles are: 79–85, 114–120, 150–154, 207–212 and 257–262. The method may not have been suited to Berkson's data, for he seems not to have used it; it is, however not very different from his second method—logit, minimum X^2—in its final result in most cases. For a discussion of the relation of the methods of maximum likelihood and minimum X^2 see Fisher, R. A., *Phil. Trans. Roy. Soc. London*, **A222**, 309–368 (1922).

[3] If we sum the deviations of the serial numbers from their mean of 3.5 over the six methods, as we do for the five in the next paragraph, we find, respectively, −10, −0.5, 0, −9.5, 1.5, 18.5. The predicted standard deviation is 5.4 and the deviation 18.5 is

quite out of the question, thus checking the simple, and often less efficient, calculation on the head-tails basis.

[4] Because of the small and variable number of figures available in the variation of the individual data from their column means and the different weights thereby implied by the different columns, no precision was carried in the arithmetical operations leading to this table which sufficed to determine precisely the unit in the last place of the entries.

VARIATION IN THE AMOUNTS OF DESOXYRIBOSE NUCLEIC ACID (DNA) IN CELLS OF THE SAME TISSUE AND ITS CORRELATION WITH SECRETORY FUNCTION

BY CECILIE LEUCHTENBERGER AND FRANZ SCHRADER

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Communicated December 10, 1951

Introduction.—It has long been known that the nuclei of the salivary gland of the snail *Helix pomatia* vary greatly in size, and that this variation is in some way correlated with the formation of secretions. Cells with large spherical nuclei show few or no secretory products in the cytoplasm whereas cells with small, irregularly shaped nuclei are characterized by large quantities of such materials. Confirming Barfurth,[1] Hirsch[7] and Krijgsmann[9] have pointed out that changes from one to the other of these types are regular and cyclical. On a cytological basis alone there is thus strong presumptive evidence that the nuclear contents are implicated in the formation of secretions. This was further strengthened by the ultraviolet studies of Caspersson[5] which demonstrated that the decrease in size of the salivary nuclei involves an actual decrease in the amount of the contained nucleic acid.

But the type of nucleic acid, whether Ribose nucleic acid (RNA) or DNA, that is concerned in these processes could not be determined by the ultraviolet method. Its determination has now become a matter of importance, for during the recent years a considerable number of investigators has claimed that the amount of DNA is constant in all the nuclei of an individual or species. It might, therefore, be supposed that in the varying amounts contained in these salivary gland nuclei we are concerned with RNA rather than DNA, but an actual identification of the nucleic acid concerned is obviously necessary. This we have proceeded to do, keeping in mind at the same time that any further information on the nature of the correlation between nuclear contents and cytoplasmic secretion products is of considerable interest.

In the present paper evidence is presented that the DNA of the cells of the *same tissue* varies in the order of magnitude of 30:1 from nucleus to

nucleus, and that such changes in the amount of DNA in individual cells are directly correlated with the secretory function of these cells.

Materials and Methods.—All work was done on the salivary glands of *Helix aspersa* Müller,[23] which were fixed in Carnoy's fluid (alcohol-acetic acid, 3:1), embedded in paraffin and cut at different thicknesses according to the requirements of the investigation. In all features at issue, the salivary glands of this species correspond to those of *Helix pomatia*.[24] The DNA content in individual cells was determined by the microspectrophotometric analysis of the Feulgen reaction as previously described.[10, 18, 22]

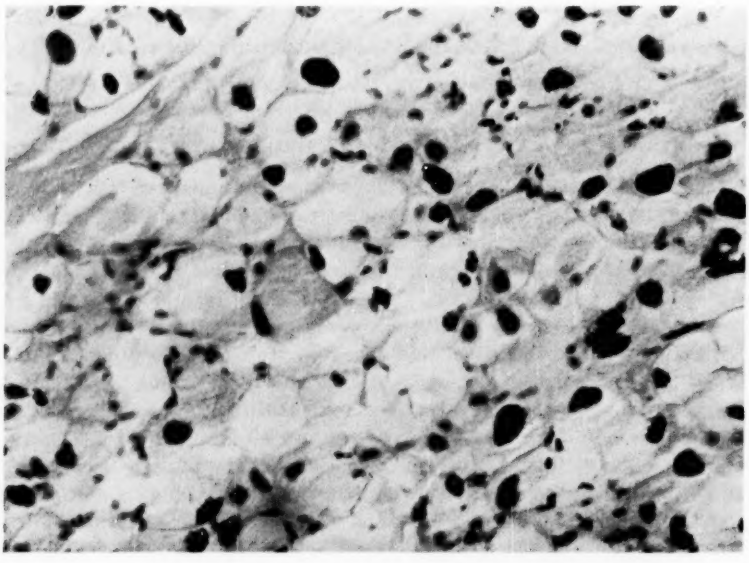

FIGURE 1

Salivary gland of *Helix aspersa* Müller stained by Feulgen-Fast Green. Magnification 330 × approx. Wratten 62.

For the estimation of the polysaccharides the PAS reaction of Hotchkiss[8] was used in conjunction with enzymes as described by Leuchtenberger and Schrader.[12]

Results.—The cytological appearance of the salivary glands of *Helix* is demonstrated in the accompanying photograph. In confirmation of the findings of Krijgsmann,[9] all stages of secretion are present within one gland. A survey, even at a low magnification (Fig. 1), of a Feulgen stained section shows the strikingly varying amounts of DNA in the different sized nuclei. A comparison of the nuclear size and amount of DNA in a non-secreting

cell with those of an actively secreting cell is so impressive in the difference displayed that it seemed hardly necessary to measure photometrically the amounts of DNA. Sections adjacent to those used for the Feulgen preparations, when stained by the PAS reaction for polysaccharides, demonstrate the relationship between nuclear size and amount of DNA on the one hand and the amounts of polysaccharides on the other hand. The larger the nucleus and the greater its content of DNA, the less polysaccharides are encountered; and the smaller the nucleus and the less DNA, the greater is the amount of polysaccharides in the cytoplasm.

In table 1 the results of the photometric measurements of DNA in individual cells are presented. It is evident from this table that the nuclei of the salivary glands of *Helix* contain widely varying amounts of DNA; the highest values are found in the non-secreting cells or in those just beginning secretion, while the lowest values are found in cells with advanced secretion. It is further obvious that there exists a direct correlation between nuclear size and amount of DNA or, in other words, the loss of DNA is accompanied by a decrease in the nuclear diameter.

The inverse relationship mentioned above between the secretion of polysaccharides and the amount of DNA is also apparent in table 1. It thus appears that the DNA is actually utilized in the synthesis of a secretory product which is of polysaccharide nature. Attempts further to identify the chemical nature of this polysaccharide have thus far shown that it is not starch, glycogen or hyaluronic acid. Perhaps we are dealing here with polysaccharides formed from pentose sugars, similar to the so-called pentosans in plants, instead of the usual hexosepolysaccharides. Testing *in situ* for the presence of pentoses in the secretory products of the salivary glands of the snails is now in progress.

It is of interest that the DNA seems to be broken down within the nucleus since in none of the cells studied was Feulgen positive material present either in the cytoplasm or in an extracellular position. These observations are in accordance with the ultra-violet studies of Caspersson,[5] who also noted that in spite of the drop of absorption at 2570 Å in the nucleus during secretion there was never an increase in the cytoplasmic absorption such as would, of course, be expected if the nucleic acid molecule were broken down only *after* leaving the nucleus.

This breakdown of the DNA molecule within the nucleus of cells which are synthesizing secretory products differs markedly from the degradation process encountered in pycnosis or necrosis. While a loss of DNA from the nucleus occurs also in such dying cells, the DNA is there found in the cytoplasm and extracellularly, which indicates that the DNA leaves the nucleus and probably even the cytoplasm, still as a complete molecule, though perhaps depolymerized.[10]

Such contrasting behavior between synthesizing and dying cells may

TABLE 1

AMOUNT OF DNA (MICROSPECTROPHOTOMETRY OF FEULGEN REACTION) IN INDIVIDUAL SECRETING AND NON-SECRETING CELLS OF THE SALIVARY GLAND OF HELIX

TYPE OF CELL	NUCLEAR DIAMETER IN MICRA	AMOUNT OF DNA PER NUCLEUS IN ARBITRARY UNITS	PRESENCE OF POLYSACCHARIDES (PAS REACTION)
Non-secretory cell "Ruhekern"	18.0 × 17.9	20.0	−
	18.1 × 17.3	20.0	−
	18.0 × 18.0	20.0	−
	18.7 × 18.5	19.0	−
	17.9 × 17.8	18.1	−
	18.9 × 18.2	17.3	−
	17.1 × 16.6	17.2	−
	20.0 × 18.1	17.3	−
	20.0 × 13.5	16.8	−
	18.9 × 13.8	16.8	−
	18.5 × 14.5	16.7	−
	18.6 × 14.3	16.7	−
	18.0 × 18.0	16.7	−
	18.8 × 14.8	16.2	−
	17.5 × 14.9	16.0	−
Beginning secretion	17.9 × 13.1	15.0	(+)
	20.7 × 13.5	15.4	(+)
	16.5 × 16.2	13.5	(+)
	16.2 × 13.4	13.4	(+)
	16.2 × 16.5	13.4	(+)
Moderate secretion	16.9 × 15.3	12.7	+
	16.2 × 13.5	11.7	+
	13.9 × 12.1	10.0	+
	14.4 × 12.6	9.7	+
	13.7 × 11.9	9.2	+
	15.1 × 11.5	8.6	+
	13.7 × 11.9	8.5	+
	13.2 × 9.6	7.7	+
	15.0 × 11.0	7.0	+
Advanced secretion	11.3 × 11.0	3.8	++++
	9.9 × 9.5	3.7	++++
	10.0 × 9.0	3.2	++++
	9.0 × 8.3	3.2	++++
	7.9 × 7.9	2.9	++++
	9.0 × 7.2	2.8	++++
	10.4 × 7.2	2.6	++++
	10.3 × 7.4	2.6	++++
	9.9 × 8.3	1.9	+++
	10.1 × 8.3	1.9	+++
	10.0 × 8.9	1.7	++++
	8.3 × 8.0	1.6	++++
	8.3 × 7.9	1.6	++++
	8.1 × 8.1	1.4	++++
	8.0 × 7.9	1.4	++++

TABLE 1—*Continued*

Advanced secretion	8.1 × 7.0	1.2	++++
	8.6 × 7.6	1.1	++++
	8.1 × 7.0	1.1	++++
	7.2 × 5.4	1.2	++++
	5.6 × 4.3	0.8	++++
	5.8 × 5.4	0.8	++++
	5.9 × 5.4	0.8	++++
	6.5 × 6.3	0.8	++++
	6.1 × 7.4	0.8	++++
	6.1 × 6.1	0.7	++++
	6.5 × 4.7	0.7	++++
	6.1 × 6.1	0.7	++++
	6.5 × 5.2	0.6	++++
	6.2 × 5.4	0.6	++++
Duct lining cells	9.4 × 4.5	3.2	−
	10.1 × 4.5	1.0	−
	10.8 × 4.5	2.2	−
	11.7 × 4.9	2.3	−
	9.4 × 5.0	1.2	−
	10.2 × 4.8	1.4	−
	10.7 × 4.6	2.4	−
	10.0 × 4.8	2.0	−
	10.0 × 4.0	1.5	−
	10.7 × 4.5	2.4	−

− = negative. (+) = doubtful. + = Small number of PAS positive secretary granules. ++++ = Large number of PAS positive secretory granules.
+++ = Large number of PAS positive secretory granules.

perhaps be regarded as supporting evidence for the view of Krijgsmann that after the maximum amount of secretory material has gathered and is extruded, this same cell then repeats the cycle of building up a nucleus and converting its content once more into secretory products. The absence of mitosis or other type of division, which might indicate that new cells are being formed to replace the spent ones, seems further to support Krijgsmann's idea of a rhythmic repetition of the process in the same cells. Nor are any replacement cells, such as are regularly found, for instance, in the basal layer of the human epidermis, ever encountered here in the salivary gland of the snail. In short, the available evidence seems to favor Krigjsmann's[9] hypothesis, though our own findings are obviously inadequate to give a final answer to this particular question.

Discussion.—The preceding analysis shows that in the great variability of nuclear size in the *Helix* salivary gland we are actually dealing with changes in the quantity of nuclear DNA. Our results, therefore, run counter to the prevailing view that DNA is constant in amount in all the nuclei of an individual or species. This view, first advanced by Boivin, Vendrely and Vendrely[2] in 1948 has since received the support of a whole series of investigations.[6, 11, 14, 22]

The present report is, however, not the first to question the universality of the constancy rule for exceptions have also been noted in certain cells of *Tradescantia*,[4, 19] as well as for various cells in embryonic tissues.[15, 16] Indeed, even in the liver of rodents which has offered some favorable evidence in support of the constancy rule, Leuchtenberger, Vendrely and Vendrely[11] have pointed out that though the *mean* amount of DNA may be the same as for all diploid nuclei of the species, measurements of *individual* nuclei may show a variation reaching 50%. It is, of course, probable that smaller departures from the constancy rule are attributable to errors inherent in the Feulgen-photometric method,[10] but the recent report of Leuchtenberger, Leuchtenberger, Vendrely and Vendrely[13] shows that the different method of ultra-violet spectrophotometry discloses a very similar range in the DNA content of individual nuclei. In short, although it is not to be contested that in the cells of some tissues, as for instance the male germ cells, there obtains a remarkable constancy of DNA, there are various conditions and types of cells where the generalization is not applicable.

Our analysis further indicates that the decrease of DNA in salivary gland nuclei is the result of its utilization in the formation of secretory products out in the cytoplasm. This secretory product is of polysaccharide nature, which raises the question how the breakdown products of DNA may be utilized for the synthesis of the polysaccharides. While in animals polysaccharides, as, for instance, glycogen, are usually formed from hexose sugar, the possibility that in the salivary gland of the snail the *pentose* sugar of DNA is utilized for the synthesis of pentose polysaccharides is worth consideration. The presence of an enzyme digesting pentose-polysaccharides within the digestive glands of *Helix*[21] seems to favor such a view. It would be intriguing indeed if the synthetic pathway of the formation of pentosans in plants, which is at present still obscure,[3] could be explained in a similar manner.

The utilization of the breakdown products of the DNA for the formation of polysaccharides might appear to be a new role for DNA, but a very similar process has also been encountered in the ovary of certain insects, such as *Acanthocephala*, by Schrader and Leuchtenberger,[20] where the products of the breakdown of DNA contribute to the nutritive materials which are transferred to the growing egg.

The prevalent view that the amount of DNA per nucleus is constant throughout the individual and species is thus evidently subject to modification and this, in turn, must affect questions concerning the deeper significance of DNA. Our investigations on *Helix* and *Acanthocephala* show that DNA may be used in the elaboration of other cellular products, which incorporates the suggestion that its role in metabolism and differentiation may be a much more direct one than is usually assumed.

Summary.—1. Evidence is presented that the different sizes of nuclei present in the salivary gland cells of *Helix* correspond to different amounts of DNA carried by them. These amounts may vary in the order of magnitude of 30:1.

2. As the size of a nucleus and its DNA content decrease, the amount of secretion in the cytoplasm increases. It appears that the DNA is actually utilized in the manufacture of such cytoplasmic secretions.

3. Since these secretory products contain polysaccharide, the possibility of formation of polysaccharides from degradation products of DNA is discussed.

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[23] We are indebted to Dr. J. Bequaert of Harvard University for the identification of this material.

[24] Material of *Helix pomatia* was collected for us by Dr. T. Caspersson, and dissected and fixed by Dr. G. Moberger and Miss U. Spilhammar of the Karolinska Institute of Stockholm, to whom grateful acknowledgment is made.

[25] We thank M. Goldstein of Western Reserve University for help in the photometric measurements.

THE PRODUCTION OF INDOLE ACETIC ACID BY USTILAGO ZEAE, AND ITS POSSIBLE SIGNIFICANCE IN TUMOR FORMATION*

By Frederick T. Wolf

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Communicated by Kenneth B. Raper, December 20, 1951

The disease known as corn smut, caused by *Ustilago zeae* (Beckm.) Ung., is characterized by the production of large galls or tumors upon various portions of affected corn plants. Studies of the pathological histology of these galls, by De Bary[1] and Knowles,[2] indicate that both hypertrophy and hyperplasia are involved in the response of the host to fungus invasion. The first insight into the mechanism of tumor production in corn smut disease was provided by Link, Wilcox and Link,[3] and by Link, Wilcox and Eggers,[4] who reported that ether extracts of cultures of *U. zeae*, grown upon media containing tryptone, gave a positive Salkowski test for indole acetic acid. Moulton[5] found that corn smut tumor tissue contained an auxin, active in tests with the *Avena* coleoptile, in much greater amounts than normal uninfected corn tissue. Auxin was detected both in the mats and in filtrates of cultures of *U. zeae*, and it was found that considerably more auxin was produced in media containing tryptone than in a synthetic medium containing neither proteins nor amino acids. No conclusion was reached as to the chemical nature of the auxin or auxins involved.

The production of indole acetic acid by a fungus was clearly demonstrated in the case of *Rhizopus suinus* Nielsen by Thimann.[6] When grown upon media containing peptone, this organism was found to produce an auxin active in the *Avena* test. It was shown by a number of chemical tests and confirmed by isolation that the auxin formed by *R. suinus* was indole acetic acid. It was also shown that tryptophane present in the peptone was the precursor of indole acetic acid, and indole pyruvic acid was suggested as a possible intermediate in this transformation.

It is the purpose of the present paper to provide further evidence indicating that the auxin produced by *U. zeae* is indole acetic acid (IAA), to demonstrate that tryptophane is the precursor of IAA in this organism, and to present evidence regarding the conversion of tryptophane to IAA. These findings lend added weight to the hypothesis that tumor formation is a direct consequence of IAA production.

Materials and Methods.—A monosporidial culture of *U. zeae* known as 17D4, provided by Dr. E. C. Stakman, was used in all experiments except when otherwise stated. Other cultures of *U. zeae* tested for comparison were sent by Dr. R. B. Stevens, and the culture of *U. nigra* Tapke was

obtained from Dr. J. G. Dickson. These isolates were grown at room temperature in a modified Czapek's solution (containing $NaNO_3$, 3.0 g.; KH_2PO_4, 1.0 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; KCl, 0.5 g.; $FeCl_3$, trace; and sucrose, 30.0 g. per liter), and in a tryptophane medium prepared by omission of sodium nitrate from the Czapek's solution, and substitution of 1.0 g. *L*-tryptophane per liter as the sole source of nitrogen. Additional details will be provided in connection with the results.

Results.—Filtrates of cultures of *U. zeae*, grown for two weeks in Czapek's solution or the tryptophane medium, were first tested for the possible presence of IAA by the Salkowski reaction. The reagent was prepared according to the directions of Berthelot, Amoreux, and Deberque.[7] Upon the addition of conc. HCl, $FeCl_3$, and boiling, a pink color is produced in the presence of IAA. The pink reaction product is soluble in *n*-butyl alcohol. When this test was applied to the filtrates of cultures grown in Czapek's medium, the results were negative. Tests with filtrates of cultures grown in the medium containing tryptophane were strongly positive for IAA.

Further tests were made using the nitrite reaction as advocated by Mitchell and Brunstetter.[8] When 0.5 ml. 5% gum arabic, 2.0 ml. 0.5% freshly prepared potassium nitrite and 0.4 ml. conc. HNO_3 are added to 50 ml. of the test solution, a cherry red color is produced by IAA or indole. Tests with filtrates of cultures of *U. zeae* on Czapek's medium were negative, while those on the tryptophane medium were again strongly positive. The *U. zeae* filtrate-nitrite reaction product was examined with a Beckman spectrophotometer, and found to have an absorption curve with a maximum near 530 mμ, indistinguishable from that given by an authentic sample of IAA upon reaction with nitrite.

Gordon and Weber[9] have pointed out a number of disadvantages of the nitrite test for IAA, including lack of specificity, low sensitivity and lack of stability of the color complex formed, and have developed a method employing ferric chloride and perchloric acid which was superior to five other methods in comparative tests. Accordingly, a reagent was prepared consisting of 1.0 ml. 0.5 *M* ferric chloride and 50 ml. 35% perchloric acid. Filtrates of cultures of *U. zeae* grown upon the tryptophane medium gave the red color characteristic of IAA when tested by this method. Further, when the filtrates of such cultures were adjusted to pH 2.0 with conc. HCl, extracted three times with an equal volume of ether, the ether removed by evaporation and the residue dissolved in water, a positive test for IAA was obtained with the resulting solution.

Because many micro-organisms produce indole, which might have been responsible for the positive findings in some of the colorimetric tests described, an experiment testing the indole production of *U. zeae* was performed by Mr. James Walton, employing the technique of Goré.[10]

Cultures of *U. zeae* were grown upon malt agar slants and in 1% Bacto-tryptone broth for two weeks. To the cotton stoppers of these tubes were applied several drops of a solution consisting of 1.0 g. *p*-dimethyl-aminobenzaldehyde, 95 ml. 95% ethyl alcohol and 20 ml. conc. HCl, as well as several drops of saturated aqueous potassium persulphate. The cotton stoppers were then pushed down into the tubes to a level about an inch above the medium, and the tubes were boiled in a water bath for 15 minutes. A red coloration on the cotton plug indicates the presence of indole. Negative results were obtained with *U. zeae*.

One-dimensional paper chromatography according to the ascending method of Williams and Kirby[11] was employed in an attempt to characterize the compound present in *U. zeae* filtrates. The solvent chosen was a 9:1 mixture of phenol and water. Filtrates of *U. zeae* cultures were extracted three times with an equal volume of ether, and the ether was evaporated to dryness. The residue was resuspended in a small quantity of water. Spots of this extract were placed near the bottom of strips of Whatman No. 1 filter paper, and the phenol-water mixture was allowed to ascend the paper strips for 12 to 15 hours. The strips were then dried and sprayed with the nitrite reagent to determine the location of the nitrite-reacting materials on the paper strip. Three spots appeared, with R_F values averaging 0.88, 0.80 and 0.70. When the paper was spotted with a sample of known IAA and treated as before, a single spot appeared at a location corresponding to R_F 0.88. When the papers were spotted simultaneously with two spots, one containing *U. zeae* filtrate alone, the other containing *U. zeae* filtrate plus IAA, it was found that the uppermost spot (R_F 0.88) was intensified in color in the latter instance. This is strong evidence that the uppermost spot in the paper chromatograms of *U. zeae* filtrates is due to IAA. No information was obtained concerning the identity of the nitrite-reacting materials responsible for the spots at R_F 0.70 and 0.80.

Since the preceding data indicate the presence of IAA in filtrates of *U. zeae* when grown in media containing tryptophane, and the absence of detectable amounts of this compound in cultures grown in Czapek's medium, experiments were performed to investigate the role of tryptophane in IAA production. When 0.5% gelatin was incorporated into media as the sole source of nitrogen for *U. zeae*, tests for the presence of IAA in the filtrate, by means of the nitrite reagent, were negative. When tryptophane was added to this medium, positive tests for IAA were obtained. Similarly, *U. zeae* does not form IAA in media in which the nitrogen is supplied as casein hydrolysate, but IAA is produced when the medium is supplemented with tryptophane.

A number of amino acids were tested for their ability to promote IAA production by *U. zeae*. Each amino acid was added to Czapek's medium

(minus $NaNO_3$) in the quantity of 100 mg. per 100 ml., and the pH was adjusted to 5.5. The amino acids tested, in addition to *L*-tryptophane, were glycine, *DL*-alanine, *DL*-serine, *DL*-threonine, *DL*-valine, *L*-aspartic acid, *L*-asparagine, *L*-glutamic acid, *L*-leucine, *DL*-isoleucine, *DL*-norleucine, *L*-cysteine·HCl, *DL*-methionine, *L*-lysine·HCl, *L*-arginine·HCl, *L*-histidine·HCl, *L*-tyrosine, *L*-proline and *DL*-phenylalanine. Results were negative for IAA production in every instance, except for tryptophane. It may therefore be concluded that tryptophane is a specific precursor in the formation of IAA by *U. zeae*.

Recent studies with higher plants, notably those of Wildman, Ferri and Bonner[12] with spinach leaves, Wildman and Bonner[13] and Larsen[14] with the *Avena* coleoptile, Galston[15] with etiolated pea seedlings and Gordon and Sanchez-Nieva[16] with pineapple, have shown the existence of two routes in the enzymatic conversion of tryptophane to IAA. One of these involves tryptamine and indole acetaldehyde as intermediates, the other involving indole pyruvic acid and indole acetaldehyde. Experiments were planned to determine which, if either, of these mechanisms was operative in *U. zeae*. It was found that tryptamine·HCl cannot serve as a sole source of nitrogen for growth of *U. zeae*. It was therefore necessary to provide nitrogen for growth in the form of nitrate, which, as previously shown, does not lead to IAA formation. Consequently, tryptamine·HCl was added, in concentrations of 50 or 100 mg. per 100 ml., to Czapek's medium, and inoculated with *U. zeae*. Although good growth resulted, tests for IAA in the filtrate by both the nitrite method of Mitchell and Brunstetter[8] and the perchloric acid method of Gordon and Weber[9] were negative. Further, when the filtrates were acidified and extracted with ether, the ether extract taken to dryness, and the residue dissolved in water, tests on the resulting solution by the perchloric acid method were negative. It is therefore apparent that tryptamine may be excluded from consideration as a possible intermediate in the transformation of tryptophane to IAA by *U. zeae*.

Attempts to demonstrate the participation of an aldehyde in the transformation of tryptophane to IAA by trapping with dimedon failed, because of reaction of the dimedon with ingredients of the Salkowski and nitrite reagents.

Any attempt to explain tumor formation as due to IAA production must take into account the possible toxicity of IAA to the inciting fungus. Richards[17] reported the inhibition of growth of *Phycomyces blakesleeanus*, *Aspergillus candidus*, *Schizophyllum commune* and *Neurospora tetrasperma* by IAA in concentrations of 10^{-2} to 10^{-3} M. Itzerott[18] found that the tolerance of *U. zeae* to IAA is very high, growth in the presence of 0.1% IAA being 98% of that in controls. Experiments with *U. zeae*, strain 17D4, upon malt agar containing 0.01% or 0.1% IAA showed that growth

in the presence of 0.01% IAA was comparable to that of untreated controls. Considerable inhibition occurred at the 0.1% concentration, but a few colonies managed to develop. The tolerance of *U. zeae* to IAA is therefore probably high enough to allow for the possibility of tumor formation without inhibition of growth of the smut.

Four monosporidial cultures of *U. zeae*, representing all the meiotic products of a single chlamydospore, were all found to produce IAA.

There are rather few smuts which induce tumor formation upon their hosts. Demonstration that smuts which fail to produce tumors do not form IAA would constitute additional evidence favoring the thesis that IAA production by *U. zeae* is related to tumor formation in corn smut disease. *U. nigra*, which causes a loose smut of barley, does not induce gall formation. When *U. nigra* was grown upon a tryptophane medium and the filtrate was tested for IAA by the nitrite and perchloric acid reagents, negative results were obtained in several trials.

Discussion.—It is known that abnormal tissue proliferations result in many species of higher plants upon application of IAA. Friedman and Francis[19] reported cellular hypertrophy, hyperplasia and disarrangement of tissue organization in tomato roots treated with IAA. In corn, Britten[20, 21] found that the application of α-naphthalene acetic acid to unpollinated ovaries resulted in parthenocarpy and abnormal differentiation of embryonic tissues.

It is believed that the production of IAA by *U. zeae* is an important factor in the development of corn smut galls. Whether the increased auxin content of corn smut galls with respect to normal corn tissue is due solely to IAA production by the parasite has not been determined. Haagen-Smit, Leech, and Bergren,[22] Berger and Avery[23] and Haagen-Smit, Dandliker, Wittwer and Murneek[24] have demonstrated by isolation the presence of IAA in corn. An increase in IAA content of corn smut tumor tissue owing to the formation of this substance by *U. zeae* may conceivably be responsible for the pathological changes observed.

In the paper chromatography of *U. zeae* filtrates, three spots were observed when the papers were sprayed with nitrite reagent, one of which was identified as IAA. The two remaining spots could not have been due to tryptophane, which gives no color with the nitrite reagent,[8] nor to tryptamine·HCl, which produces a faint yellow color. Although proof is lacking, it would appear probable that the intermediates in the formation of IAA from tryptophane by *U. zeae* are indole pyruvic acid and indole acetaldehyde.

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tion Board to Mr. William T. Allman, Jr., which made possible his assistance with the experiments.

Summary.—Indole acetic acid has been identified as a metabolic product of *Ustilago zeae*. The identification of this compound is based upon three independent color tests, and upon identity of R_F values obtained by paper chromatography. Tests with gelatin, casein hydrolysate and a number of individual amino acids indicate that tryptophane is the precursor of IAA in *U. zeae*. The mechanism of conversion of tryptophane to IAA is not known with certainty, but it is shown that tryptamine cannot be an intermediate.

U. nigra, a smut which does not form tumors upon its host, failed to produce from tryptophane a quantity of IAA detectable by colorimetric tests. The present findings add weight to the theory that IAA production by *U. zeae* is important in the development of the tumors characteristic of corn smut disease.

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THE STRUCTURE OF CHLORINE HYDRATE

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In 1811 Humphry Davy[1] showed that water is a component of the phase that had earlier been thought to be solidified chlorine, and twelve years later Michael Faraday[2] reported an analysis that corresponds to the formula $Cl_2 \cdot 10H_2O$. He surmised that his determination of the chlorine content was low, and later studies have indicated the composition to be close to $Cl_2 \cdot 8H_2O$. Since Faraday's time similar crystalline hydrates of many gases with small molecular volume, including the noble gases and simple hydrocarbons, have been reported. The determination of the structure of ice and the development of an understanding of the nature of the hydrogen bond have strongly suggested that these substances are clathrate compounds, with a tetrahedral hydrogen-bonded framework of water molecules (with O—H $\cdots$ O = 2.76 A, as in ice) defining cavities large enough to contain the other molecules.

Only recently have serious steps been taken toward determining the structure of these hydrates. About ten years ago von Stackelberg and his collaborators[3,4] made x-ray studies of some of these compounds, including the hydrates of xenon, chlorine, bromine, sulfur dioxide, hydrogen sulfide, methyl bromide, methyl iodide, ethyl chloride and chloroform. They reported that most of the crystals have a cubic unit cell with a_0 equal to about 12.0 A. From single-crystal x-ray photographs of the hydrate of sulfur dioxide they decided on the space group O_h^3, and postulated a structure consisting of a framework of 48 oxygen atoms in a set of general positions x, y, z in the unit cell. With this structure each oxygen atom has neighboring oxygen atoms at a distance of about 2.4 A, and the framework has eight cavities in which the gas molecules could lie. The ideal formula would thus be $M \cdot 6H_2O$. This structure is not acceptable because of the very short O—H $\cdots$ O distance of 2.4 A; in addition, the hydrogen bonds lie at angles between 60° and 145°, which differ greatly from the expected tetrahedral angle.

Claussen[5] then proposed a structure based on a larger cubic unit cell, about 17 A on edge, containing 136 water molecules. The oxygen atoms would be arranged to form 16 pentagonal dodecahedra and 8 hexakaidecahedra. This structure leads to the empirical formula $M \cdot 5^2/_3 H_2O$ for smaller molecules which occupy all of the polyhedra, and $M \cdot 17H_2O$ for larger molecules which occupy only the hexakaidecahedra. The obvious advantage of such a structure is the presence of hydrogen bond angles of

about 108° in the dodecahedra and in the twelve pentagonal faces of the hexakaidecahedra; the other hydrogen bond angles, in the four hexagonal faces of the hexakaidecahedra, are about 120°. Von Stackelberg and Müller[6] have recently reported evidence for the existence of this structure derived from analysis of x-ray diffraction photographs of alkyl halide hydrates.

We have been studying the structures of intermetallic compounds that involve icosohedral and pentagonal dodecahedral arrangements of atoms, and in the course of this work a structure was found which seemed to be ideally suited to the hydrates of smaller gas molecules. This structure is based on the cubic group O_h^3, and has a_0 equal to 11.88 A for the O—H $\cdots$ O distance 2.76 A, in close agreement with the first results reported by von Stackelberg. The unit cell contains 46 water molecules, with the oxygen atoms arranged so as to form two pentagonal dodecahedra and six tetrakaidecahedra; the empirical formula is hence $M \cdot 7^2/_3 H_2O$ for molecules M occupying only the tetrakaidecahedra, and $M \cdot 5^3/_4 H_2O$ for smaller molecules which occupy all of the polyhedra.[7] In order to test the predicted structure we have prepared and interpreted x-ray diffraction photographs of chlorine hydrate.

Experimental Methods and Results.—A length of 6-mm. Pyrex tubing was drawn to a capillary at one end and connected to a tank of commercial chlorine. After thorough flushing, the capillary was sealed off and the end was immersed in a dry-ice–acetone bath. When some chlorine had condensed in the capillary a drop of water was admitted into the large end of the glass tube, which was then sealed off. Alternate warming and cooling of the capillary permitted thorough mixing of the water and chlorine. Small pale-yellow crystals soon formed in the capillary, and remained as the temperature was brought up to 0°C. A small amount of liquid chlorine also remained, showing the chlorine to be in excess and indicating a pressure of about 4 atmospheres.

The Pyrex tube was suspended, with capillary down, in a small-holed rubber stopper which, in turn, was fastened to a goniometer head by a length of stout copper wire. The solid material within the capillary was photographed in a cold room (4°C.) using copper x-radiation, a camera with radius 5 cm., and oscillation range 30°. The effective camera radius was established by superimposing a powder spectrum of NaCl during an exposure of the sample; the lattice constant for NaCl at 4°C. was taken to be 5.634 A.

The diffraction pattern of the sample of chlorine hydrate consisted of powder lines on which were superimposed a large number of more intense single-crystal reflections; for some planes only the latter were visible. The intensities of the lines were estimated by comparison with a previously calibrated powder photograph, and were averaged for several films pre-

pared from different parts of the capillary (and which, accordingly, varied as to the intensities of the single-crystal reflections). Despite this precaution, it was expected that the intensity estimations would be subject to considerably larger errors than would a properly ground powder sample. The averaged intensities were corrected for Lorentz and polarization factors and were adjusted by an empirical scale factor to give values of G^2_{obs}.

All of the observed reflections could be indexed on the basis of a cubic unit cell with $a_0 = 11.82$ A; the estimated probable error is 0.01 A. The only systematic absences were *hhl* with *l* odd; this is characteristic of the space group O_h^3-$Pm3n$, which also was reported by von Stackelberg from his single-crystal work on sulfur dioxide hydrate. For 46 H_2O and 6 Cl_2 in the unit cell the calculated density is 1.26; densities reported by various observers range from 1.23 to 1.29.

In order for all of the O—H $\cdots$ O distances within the unit cell to be the same the oxygen atoms must lie in the following positions:

$$6\ O_I \text{ in } 6(c)\ \frac{1}{4}\ 0\ \frac{1}{2}\ \ldots;$$

$$16\ O_{II} \text{ in } 16(i)\ xxx,\ \ldots,\ x = 0.183;$$

$$24\ O_{III} \text{ in } 24(k)\ 0yz,\ \ldots,\ y = 0.310,\ z = 0.116.$$

For comparison with G^2_{obs} values, calculated values were determined as follows: $G^2_{calc} = \sum_i m_i F_i^2$ where F_i is the structure factor and m_i the multiplicity of each set of planes i contained in the powder line. For preliminary calculations only the contributions of the oxygen atoms were included in the structure factor; nevertheless, there was rough agreement with the observed G^2 values.

The oxygen atoms placed in the above positions form, in each unit cell, a framework consisting of two pentagonal dodecahedra, centered at (000) and $\left(\frac{111}{222}\right)$, and six tetrakaidecahedra, centered at $\left(\frac{11}{42}0\right)$, $\left(\frac{31}{42}0\right)$, $\left(\frac{1}{2}0\frac{1}{4}\right)$, $\left(\frac{1}{2}0\frac{3}{4}\right)$, $\left(0\frac{11}{42}\right)$, and $\left(0\frac{31}{42}\right)$. It soon became apparent that the agreement between calculated and observed values of G^2 was markedly improved if the chlorine molecules were placed within the six tetrakaidecahedra only, leaving the dodecahedra empty. These tetrakaidecahedra have two nearly regular hexagonal faces at opposite ends, separated by twelve nearly regular pentagons, as shown in figure 1. A cross-section taken parallel to the hexagonal faces is nearly circular, and the equatorial diameter is considerably larger than the polar diameter (between hexagons). The figure thus is a close approximation to an oblate spheroid. If the twelve chlorine atoms are to lie in fixed positions within the tetrakaidecahedra conforming to the crystal symmetry, a pair must lie along the short polar

axis. This arrangement is sterically unfavorable, and calculations based on it gave rather poor intensity agreement. Since the tetrakaidecahedra containing the chlorine molecules so nearly approximate spheroids, it was

TABLE 1

CALCULATED AND OBSERVED G^2 VALUES FOR CHLORINE HYDRATE

	$mF^2_{calc.}$				
hkl	(I) UNIFORM SHELL	(II) EQUAT. CIRCLE	(III) WEIGHTED SHELL	(IV) V. STACKEL-BERG	$G^2_{obs.}$
110	555	488	527	34	207
200	10	18	0	722	<48
210	865	986	916	278	636
211	8	4	1	221	<74
220	412	809	562	3204	582
310	981	602	808	23	694
222	4390	4283	4390	1656	2732
320	4195	3120	3741	2307	3394
321	6416	6037	6304	723	9216
400	970	1083	972	207	679
410	1579	4494	2528	60	1817
330	522	844	653	83	1074
411	274	1142	525	292	
420	94	389	167	2679	139
421	246	16	117	3	323
332	429	666	509	357	318
422	4	528	107	1144	141
430	346	0	110	2323	206
431	37	299	128	1354	229
510	330	80	194	128	
432	1129	1260	1109	701	2411
520	180	5	42	2949	
521	161	493	272	46	196
440	0	4	0	631	<183
433	2401	2599	2578	1144	10080
530	5040	3863	4437	1234	
531	2611	2611	2611	10	1374
442	484	955	730	1080	1020
600	1568	274	957	234	
610	1152	2	459	750	304
532	5685	4264	5069	10	5236
611	392	2035	764	99	
620	960	2964	1562	359	1320
540	620	1008	890	5317	1654
621	296	3192	973	1290	
541	49	85	37	491	<378
622	166	95	164	689	<400

decided to treat the chlorine molecules as freely rotating about their centers or else as being statistically distributed over a large number of orientations within the polyhedra. In either case, a sufficiently close

approximation to the electron density is given by considering the centers of the chlorine atoms to lie on spherical shells concentric with the tetrakaidecahedra; the diameter of these shells is the Cl—Cl bonded distance, taken as 1.98 A. Since the tetrakaidecahedra are oblate, it would be expected that the chlorine atoms would be concentrated toward the equator. Calculations of structure factors were made on the basis of three different configurations for the chlorine atoms within each tetrakaidecahedron: (I) a spherical shell of uniform density; (II) the limiting case in which all of the chlorine atoms were assumed to lie on the equator; that is, on a circle parallel to and midway between the hexagonal faces; (III) a spherical shell with density weighted according to the function $\cos^2 \alpha$, where α is the angle of latitude. For arrangement I the calculated structure factor for the center of the sphere, including the form factor for two chlorine atoms, was multiplied by the factor $\frac{\sin P}{P}$, where $P = \frac{2\pi r}{d_{hkl}}$, d_{hkl} is the spacing of the diffracting plane, and r (= 0.99 A) is the radius of the sphere. For the circle in arrangement II the factor is J_0 ($P \sin \psi$), where J_0 is the zero-order Bessel coefficient and ψ is the angle between the axis of the circle and the normal to the diffracting plane *hkl*. Arrangement III was approximated by summing up the appropriately weighted contributions of circles with radius $r \cos \alpha$, taken at each 15° of latitude; the calculated structure factor for the center of each circle was multiplied by the correction factor J_0 ($P \cos \alpha \sin \psi$). As a check that 15° was a sufficiently small interval, another calculation was made in which the latitudinal circles were weighted so as to give an approximation to a uniform spherical shell; the results were essentially identical with those of arrangement I throughout the observed range of diffraction angle.

In table 1 there are listed the values of G^2 calculated on the basis of these three arrangements, together with the observed values; it is seen that arrangement III—the spherical shell of non-uniform density—gives the best agreement, especially for the reflections 430, 610, and 540 + 621. Indeed, the agreement with the observed values is quite satisfactory in view of the relatively large uncertainty in observed intensities. Contributions of the hydrogen atoms were not included in the calculations, and no temperature factor was applied. The reliability factor $R^2 = \frac{\sum |G^2_{obs.} - G^2_{calc.}|}{\sum G^2_{obs.}}$ has a value of 0.32, which corresponds to a value of 0.16 if F values were used.

In column IV there are listed the G^2 values calculated from the parameters proposed by von Stackelberg. As an approximation to the configuration of the chlorine molecules spherical shells of uniform density were placed at the centers of the eight cavities formed by the oxygen atoms. It is seen that the calculated values are not compatible with

the observed ones, and it seems extremely unlikely that any change in the positions of the chlorine atoms would make the agreement between calculated and observed intensities satisfactory.

Discussion of the Structure.—Figures 1 and 2 show two different representations of the framework of oxygen atoms. The structure may be derived, as shown in figure 2, by placing dodecahedra at the corners and body center of the unit cube and adding six additional atoms per unit cell (three of these are indicated by circles) to form the tetrakaidecahedra. Each dodecahedron shares its faces with twelve surrounding tetrakaidecahedra, and each tetrakaidecahedron shares its two hexagonal faces and eight pentagonal faces with neighboring tetrakaidecahedra, the remaining four pentagonal faces being shared with dodecahedra. With the oxygen parameters given above, which result in O—H$\cdots$O distances of 2.75 A

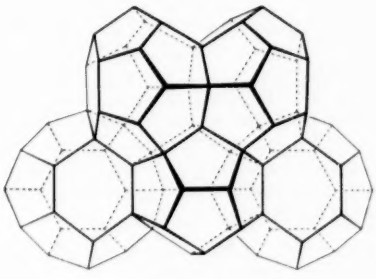

FIGURE 1

Dodecahedron of oxygen atoms surrounded by four out of a total of twelve tetrakaidecahedra.

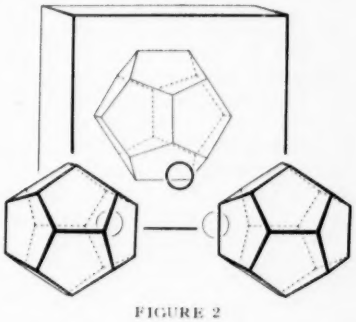

FIGURE 2

Dodecahedra of oxygen atoms at two corners and body center of unit cell, with three additional oxygen atoms (circles) needed to complete the structure.

throughout, the pentagons deviate slightly from planarity, while the hexagons are planar but not equi-angular. The O-O-O angles on the pentagonal faces range from 106.1° to 114.8°, while each hexagon has two angles of 109.6° and four of 125.2°.

The average radius of the tetrakaidecahedra at the equator is 4.46 A, while the average distance from the center to the six vertices of the hexagonal faces is 4.03 A; the average radius of the dodecahedra is 3.85 A. Using a single-bond radius for chlorine of 0.99 A and van der Waals radii for chlorine and oxygen of 1.80 and 1.40 A, respectively, we would expect a minimum radius of 4.19 A for accommodation of a chlorine molecule. Even if the presence of hydrogen-chlorine contacts would decrease somewhat the effective van der Waals radius of chlorine, it is doubtful that the over-all minimum radius could drop to 3.85 A; hence one would predict

that the dodecahedra are too small to accommodate the chlorine molecules. Moreover, the tetrakaidecahedra are barely long enough to hold the chlorine molecules along the polar axis; the $\cos^2 \alpha$ weighting of the density of the spherical shells representing the centers of the chlorine atoms thus appears to be justified.

On the basis of this structure for chlorine hydrate, the empirical formula is $6Cl_2 \cdot 46H_2O$, or $Cl_2 \cdot 7^2/_3 H_2O$. This is in fair agreement with the generally accepted formula $Cl_2 \cdot 8H_2O$, for which Harris[7] has recently provided further support. For molecules slightly smaller than chlorine, which could occupy the dodecahedra also, the predicted formula is $M \cdot 5^3/_4 H_2O$.

[*] Contribution No. 1652.

[1] Davy, H., *Phil. Trans. Roy. Soc.*, **101**, 155 (1811).

[2] Faraday, M., *Quart. J. Sci.*, **15**, 71 (1823).

[3] *Fiat Review of German Science*, Vol. 26, Part IV.

[4] v. Stackelberg, M., Gotzen, O., Pietuchovsky, J., Witscher, O., Fruhbuss, H., and Meinhold, W., *Fortschr. Mineral.*, **26**, 122 (1947); cf. *Chem. Abs.*, **44**, 9846 (1950).

[5] Claussen, W. F., *J. Chem. Phys.*, **19**, 259, 662 (1951).

[6] v. Stackelberg, M., and Müller, H. R., *Ibid.*, **19**, 1319 (1951).

[7] In June, 1951, a brief description of the present structure was communicated by letter to Prof. W. H. Rodebush and Dr. W. F. Claussen. The structure was then independently constructed by Dr. Claussen, who has published a note on it (*J. Chem. Phys.*, **19**, 1425 (1951)). Dr. Claussen has kindly informed us that the structure has also been discovered by H. R. Müller and M. v. Stackelberg.

[8] Harris, I., *Nature*, **151**, 309 (1943).

ON THE HOMOMORPHISM THEOREM FOR SEMIRINGS

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Communicated by F. D. Murnaghan, December 10, 1951

In a recent paper,[1] we have given a statement of the homomorphism theorem for semirings,[2] which is in part incorrect. We have noted this in these PROCEEDINGS, **37**, 461 (1951). At present, it is our purpose to give and prove a corrected statement of this theorem.

Definition: A semiring S is said to be semi-isomorphic to the semiring S', if S is homomorphic to S' and the kernel of this homomorphism is (0).

THEOREM. *If the semiring S is homomorphic to the semiring S', then the difference semiring $S - I$ is semi-isomorphic to S', where I is the ideal of elements mapped onto $0'$.*

Proof: The correspondence $\bar{s} \rightarrow s'$, where s' is the image of an element of the coset $\bar{s}$, is a semi-isomorphism of $S = S - I$ into S'. The correspondence is certainly a homomorphism, for if $\bar{s} = (s_1, s_2, \ldots)$, then $s_1 + i_1 = s_2 + i_2$, i_1 and i_2 in I[1], and $(s_1 + i_1)' = s_1' = (s_2 + i_2)' = s_2'$. $\bar{s} + \bar{t} \rightarrow s' + t'$ and $\bar{s}\bar{t} \rightarrow s't'$ follow from coset addition and multiplication. If $\bar{s} \rightarrow 0'$, then $s_1 + i_1 = s_2 + i_2$, i_1 and i_2 in I, and $s_1' = s_2' = 0'$. Thus s_1 and s_2 are both contained in I. This implies that the kernel of the homomorphism $\bar{s} \rightarrow s'$ is $\bar{0}$ and this homomorphism is a semi-isomorphism.

Previously,[1] we have proved the converse of the above theorem. Hence, we may state *the homomorphism theorem for semirings* as

THEOREM: *If I is an ideal of S, then S is homomorphic to the difference semiring $S - I$. Conversely, if the semiring S is homomorphic to the semiring S', then the difference semiring $S - I$ is semi-isomorphic to S', where I is the ideal of elements mapped onto $0'$.*

[1] Bourne, S., "The Jacobson Radical of a Semiring," PROC. NATL. ACAD. SCI., **37**, 163 (1951).

[2] Vandiver, H. S., "Note on a Simple Type of Algebra in Which the Cancellation Law of Addition Does Not Hold," *Bull. Am. Math. Soc.*, **40**, 920 (1934).

A NOTE ON $\pi_r(V_{n,m})$

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Introduction.—Let $k \geq 3$. We shall prove

THEOREM 1.1. *$\pi_{k+3}(S^k)$ has an element of order four.*

Let $V_{k+m,\,m}$ be the Stiefel Manifold of all orthogonal m-frames in real Euclidean $(k + m)$-space.

THEOREM 1.2. *The groups $\pi_{k+2}(V_{k+m,\,m})$ are given by the following table, in which Z_p, Z_∞, are cyclic groups of order p, ∞, respectively.*

$\pi_{2k,\,m}$	$m = 1$	$m = 2$	$m = 3$	$m \geq 4$
$k = 1$	0	Z_∞	$Z_\infty + Z_\infty$	Z_∞
$k = 4s - 2$	Z_2	$Z_2 + Z_2$	Z_2	0
$k = 4s$	Z_2	$Z_2 + Z_2$	$Z_2 + Z_2$	$Z_2 + Z_2$
$k = 4s - 1$	Z_2	Z_4	$Z_2 + Z_\infty$	Z_2
$k = 4s + 1$	Z_2	Z_4	$Z_4 + Z_\infty$	Z_4

Let Y^{n+1} be the $(n - 1)$-fold suspension of the real projective plane, so that Y^{n+1} consists of an n-sphere S^n and an $(n + 1)$-cell e^{n+1} attached to S^n by a map of degree 2. We prove

THEOREM 1.3 *$\pi_{n+2}(Y^{n+1}) = Z_4$ if $n \geq 3$.*

Proof of 1.1 and 1.3:[1] Let $\mu: Y^{n+1} \to S^{n+1}$ be a map which shrinks S^n to a point and maps e^{n+1} homeomorphically onto $S^{n+1} - \mu(S^n)$. Then Hilton[2] has shown by suspension methods that the induced homomorphism $\mu_*: \pi_{n+2}(Y^{n+1}) \to \pi_{n+2}(S^{n+1})$ is onto when $n \geq 3$. Therefore there is a map $\nu: S^{n+2} \to Y^{n+1}$ such that $\mu\nu$ is essential. Consider the sequence

$$S^{n+2} \xrightarrow{\nu} Y^{n+1} \xrightarrow{\mu} S^{n+1} \xrightarrow{\lambda} S^{n-1},$$

in which λ is an essential map. Hilton[2] has shown that the composition $\lambda\mu\nu$ is essential when $n \geq 4$.

When $n \geq 2$, the homotopy classes $\{g\}$ of maps $g: Y^{n+1} \to S^{n-1}$ are the elements of a *Track Group*, which is the cohomotopy group $\pi^{n-1}(Y^{n+1})$ when $n \geq 5$. One of us[3] has shown that this group is cyclic of order four when $n \geq 4$. Moreover $\{f\}$ is one of the generators if and only if $f \mid S^n$ is an essential map. Since $\mu(S^n)$ is a point, $\lambda\mu \mid S^n$ is inessential, so that there is a map $f: Y^{n+1} \to S^{n-1}$ when $n \geq 4$, such that $2\{f\} = \{\lambda\mu\}$, and $4\{f\} = 0$.

It is easily verified by suspension methods[4] that the suspension homomorphism $\mathfrak{E}: \pi_{n+1}(Y^n) \to \pi_{n+2}(Y^{n+1})$ is an isomorphism onto when $n \geq 4$. Hence $\nu: S^{n+2} \to Y^{n+1}$ is homotopic to the suspension of a map $\nu': S^{n+1} \to Y^n$. Therefore[5] ν induces a homomorphism ν^* of this Track Group into $\pi_{n+2}(S^{n-1})$, defined by setting $\nu^*\{g\} = \{g\nu\}$. Hence

$$\begin{aligned} 2\{f\nu\} &= 2\nu^*\{f\} = \nu^*(2\{f\}) = \nu^*\{\lambda\mu\} = \{\lambda\mu\nu\} \neq 0, \\ 4\{f\nu\} &= 4\nu^*\{f\} = \nu^*(4\{f\}) = 0, \end{aligned}$$

so that $\{f\nu\} \in \pi_{n+2}(S^{n-1})$ is of order four if $n \geq 4$. This proves 1.1.

Also

$$0 \neq 2\{f\nu\} = 2f_*\{\nu\} = f_*(2\{\nu\})$$

so that $2\{\nu\} \neq 0$ in $\pi_{n+2}(Y^{n+1})$ if $n \geq 4$. But it is known that this group has four elements.[6] Therefore $\pi_{n+2}(Y^{n+1}) = Z_4$ $(n \geq 4)$, while $\mathfrak{E}: \pi_5(Y^4) \approx \pi_6(Y^5)$. This proves 1.3.

Proof of 1.2: In order to prove Theorem 1.2 it is sufficient to modify certain arguments of Eckmann[7] and J. H. C. Whitehead,[8] in which it is assumed that $\pi_5(S^3) = 0$. The only outstanding problem of any difficulty is the determination of $\pi_{k+2}(V_{k+2,\,2})$ when k is odd. Let P^n_{k-1} mean the same as in Whitehead's paper. Then $P^{k+1}_{k-1} = Y^{k+1}$ if k is odd, and, by his Theorem 3, $\pi_{k+2}(V_{k+2,\,2}) \approx \pi_{k+2}(P^{k+1}_{k-1})$ if $k \geq 3$.

There is now no difficulty in calculating $\pi_{k+2}(V_{k+m,\,m})$. For example, one can proceed by induction on m, with the help[9] of the exact sequence $\ldots \to \pi_{k+2}(V_{k+m,\,m}) \to \pi_{k+2}(V_{k+m+1,\,m+1}) \to \pi_{k+2}(S^{k+m}) \to \ldots$, associated with the fibring $V_{k+m+1,\,m+1} / V_{k+m,\,m} \to S^{k+m}$.

[1] We are deeply grateful to J. H. C. Whitehead and P. J. Hilton, to whom this proof, a simplification of an earlier proof, is due.

[2] See a forthcoming paper by Hilton.

[3] See §9, 10 in a forthcoming paper on Track Groups by M. G. Barratt, concerning the homotopy groups of function spaces X^P. The group in question is

$$\pi_{n-2}\,[(S^{n-1})^{Y^2}, x_0]$$

where x_0 is an inessential map.

[4] Whitehead, J. H. C., "A Note on Suspension," *Q. J. Math.* (*Oxford*) (2), **1**, 9–22 (1950). See also reference 2.

[5] See reference 3, §2. A map $\phi: P \to Q$ induces a homomorphism $\phi^*: \pi_k(X^Q) \to \pi_k(X^P)$ for a large class of spaces P, Q, X.

[6] Hilton, P. J., "The Calculation of Homotopy Groups of A_n^2-polyhedra (II)," *Ibid.*, (2), **2**, 228–240 (1951). Theorem 1.3 settles the problems stated on pages 234 and 240.

[7] Eckmann, B., "Zur Homotopietheorie Gefaserter Räume," *Comm. Math. Helvetici*, **14**, 141–192 (1941); also "Uber Homotopiegruppen fon Gruppenräumen," *Ibid.*, **14**, 234–256 (1941).

[8] Whitehead, J. H. C., "On the Groups $\pi_r(V_{n,m})$ and Sphere Bundles," *Proc. London Math. Soc.*, **48**, 243–291 (1943).

[9] The key theorems of Eckmann and Whitehead are Theorem 12 in reference 8, and Theorems 7, 9 and the construction on pages 270–271 in reference 9.

*FIXED-POINT AND MINIMAX THEOREMS IN LOCALLY CONVEX TOPOLOGICAL LINEAR SPACES**

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Communicated by John von Neumann, December 13, 1951

1. Let K be a compact convex set in a Euclidean space and $\mathfrak{K}(K)$ be the family of all closed convex (non-empty) subsets of K. Kakutani's fixed-point theorem[1] asserts that, for any upper semicontinuous point-to-set transformation f from K into $\mathfrak{K}(K)$, there exists a point $x_0 \in K$ such that $x_0 \in f(x_0)$. Here upper semicontinuity means that $\lim x_n = x_0$, $y_n \in f(x_n)$ and $\lim y_n = y_0$ imply $y_0 \in f(x_0)$. On the other hand, A. Tychonoff[2] has shown that, if K is a compact convex set in a locally convex topological linear space L, then any continuous point-to-point transformation from K into itself has at least one fixed point. In the present note, Theorem 1 is a common generalization of both theorems of Kakutani and Tychonoff. Like Tychonoff, we consider a compact convex set K in a locally convex topological linear space L. Like Kakutani, we deal with upper semicontinuous point-to-set transformations from K into $\mathfrak{K}(K)$. However, since the first countability axiom is not assumed for the space L, we have to define the upper semicontinuity in terms of open sets, not in terms of convergent sequences. (The two definitions are equivalent for metric spaces.) Recently H. F. Bohnenblust and S. Karlin[3] have extended Kakutani's theorem to Banach spaces along the same lines as Schauder's fixed-point theorem[4] generalizes Brouwer's; their result does not include Tychonoff's theorem.

Theorems 2 and 3 of this note are applications of Theorem 1. In the case of two Euclidean spaces, Theorem 2 was first proved by von Neumann.[5] Theorem 3 extends von Neumann's minimax theorem[6] to locally convex topological linear spaces, and thus includes also the minimax theorem of J. Ville.[7]

2. As a topological linear space is not always metrizable, but is always a separated uniform space,[8] we shall need the following

LEMMA 1. *Let X be a separated uniform space. Let A be a closed set and B a compact set in X. For any entourage U of the uniform structure of X, there exists another entourage V such that*

$$V(A) \cap V(B) \subset U(A \cap B), \tag{1}$$

where $V(A) = \{x \in X | (a, x) \in V \text{ for some } a \in A\}$ and $V(B)$, $U(A \cap B)$ have similar meaning.

Proof: Choose an open entourage U_1, such that $U_1^2 \subset U$. Then $U_1(A \cap B)$ is open. Let C be the set of all points of B not belonging to $U_1(A \cap B)$. Then C is a compact set disjoint from the closed set A. We can find an entourage U_2 such that $C \cap U_2(A) = \phi$, which implies $B \cap U_2(A) \subset U_1(A \cap B)$. If V is an entourage such that $V = V^{-1} \subset U_1$ and $V^2 \subset U_2$, then (1) is satisfied. In fact, for any $x \in V(A) \cap V(B)$, we have $(a, x) \in V$, $(b, x) \in V$ with $a \in A$, $b \in B$. Since $V = V^{-1}$, we have $(a, b) \in V^2 \subset U_2$ and therefore $b \in B \cap U_2(A) \subset U_1(A \cap B)$. There exists $c \in A \cap B$ such that $(c, b) \in U_1$. This together with $(b, x) \in V$ implies $(c, x) \in V \cdot U_1 \subset U_1^2 \subset U$ or $x \in U(c) \subset U(A \cap B)$.

Let X, Y be two topological spaces and let $\mathfrak{C}(Y)$ denote the family of all closed sets in Y. A point-to-set transformation f from X into $\mathfrak{C}(Y)$ is called *upper semicontinuous* (u. s. c.), if, for any point $x_0 \in X$ and any open set U in Y such that $f(x_0) \subset U$, there exists a neighborhood W of x_0 such that $f(x) \subset U$ for all $x \in W$.

THEOREM 1. *Let L be a locally convex topological linear space[9] and K a compact convex set in L. Let $\mathfrak{K}(K)$ be the family of all closed convex (non-empty) subsets of K. Then for any u. s. c. point-to-set transformation f from K into $\mathfrak{K}(K)$, there exists a point $x_0 \in K$ such that $x_0 \in f(x_0)$.*

Proof: Let $\mathfrak{V}$ be a fundamental system of neighborhoods of the null-element 0 in L. L being locally convex, we may assume that each $V \in \mathfrak{V}$ is convex, open and such that $V = -V$. For each $V \in \mathfrak{V}$, let

$$F_V = \{x \in K | x \in f(x) + \bar{V}\}.$$

If we can prove that each F_V is non-empty and closed, then it will follow that also any finite intersection of them is non-empty. The compactness

of K will then imply $\bigcap_{V \in \mathfrak{V}} F_V \neq \phi$. Clearly any point x_0 in this intersection will satisfy $x_0 \in f(x_0)$.

Consider an arbitrarily fixed $V \in \mathfrak{V}$. As K is compact, there exists a finite number of points $z_1, z_2, \ldots, z_n$ of K such that $K \subset \bigcup_{i=1}^{n} (V + z_i)$. Let C be the smallest closed convex set containing $z_1, z_2, \ldots, z_n$. Let $\mathfrak{K}(C)$ be the family of all closed convex (non-empty) subsets of C. For each $x \in C$, we define

$$f_V(x) = (f(x) + \bar{V}) \cap C.$$

Since V is convex, it can be easily seen that f_V is a transformation from C into $\mathfrak{K}(C)$. We claim that f_V is u. s. c. Let $x_0 \in C$ and let U be an open set in L such that $f_V(x_0) \subset U$. As $f_V(x_0)$ is compact, we can find a $V_1 \in \mathfrak{V}$ such that $V_1 + f_V(x_0) \subset U$. By Lemma 1, there exists a $V_2 \in \mathfrak{V}$ such that

$$(V_2 + f(x_0) + \bar{V}) \cap (V_2 + C) \subset V_1 + [(f(x_0) + \bar{V}) \cap C].$$

We have then

$$(V_2 + f(x_0) + \bar{V}) \cap C \subset V_1 + f_V(x_0) \subset U.$$

Now, f being u. s. c., there is a neighborhood W of x_0 such that $f(x) \subset V_2 + f(x_0)$ for all $x \in W \cap K$. Hence, for $x \in W \cap C$, we have

$$f_V(x) = (f(x) + \bar{V}) \cap C \subset (V_2 + f(x_0) + \bar{V}) \cap C \subset U.$$

This proves that f_V is u. s. c. By Kakutani's theorem, there is a point $x_0 \in C$ such that $x_0 \in f_V(x_0) \subset f(x_0) + \bar{V}$. Hence $F_V \neq \phi$.

Let $y \in K$ be not contained in F_V. Then y is not contained in the closed set $f(y) + \bar{V}$. There is a $V_3 \in \mathfrak{V}$ such that

$$(y + V_3) \cap (f(y) + \bar{V} + V_3) = \phi.$$

Again since f is u. s. c., there is a $V_4 \in \mathfrak{V}$ such that $f(z) \subset f(y) + V_3$ for all $z \in (y + V_4) \cap K$. We may assume $V_4 \subset V_3$. Then for any $z \in (y + V_4) \cap K$, z is not in $f(z) + \bar{V}$, nor in F_V. This shows that F_V is closed and completes the proof.

3. LEMMA 2. *Let X, Y be two topological spaces, of which Y is compact. Let E be a closed set in the product space $X \times Y$. For any $x \in X$, let $E(x)$ be the section of E determined by x: $E(x) = \{y \in Y \mid (x, y) \in E\}$. Then $x \to E(x)$ is an u. s. c. transformation from X into $\mathfrak{C}(Y)$.*

Proof: For any subset A of X, we use the notation $E(A) = \bigcup_{a \in A} E(a)$. Let $\mathfrak{W}$ be the family of all neighborhoods of a point $x_0 \in X$. Then we have $E(x_0) = \bigcap_{W \in \mathfrak{W}} \overline{E(W)}$. Now, if U is an open set in Y containing $E(x_0)$, and if F denotes the complement of U in Y, then $F \cap \left[\bigcap_{W \in \mathfrak{W}} \overline{E(W)}\right] = \phi$, and,

since Y is compact, there exists a finite number of neighborhoods W_1, W_2, ..., W_n of x_0 such that $F \cap \left[\bigcap_{i=1}^{n} \overline{E(W_i)}\right] = \phi$. Thus $W = \bigcap_{i=1}^{n} W_i$ is a neighborhood of x_0 such that $E(W) \subset U$.

LEMMA 3. *Let X be a topological space and $\{Y_\nu\}_{\nu \in I}$ be a family of compact spaces. Let $Y = \prod_{\nu \in I} Y_\nu$ be the product space. If, for each $\nu \in I$, f_ν is an u. s. c. transformation from X into $\mathfrak{C}(Y_\nu)$, then the transformation f from X into $\mathfrak{C}(Y)$ defined by $f(x) = \prod_{\nu \in I} f_\nu(x)$ is also u. s. c.*

Proof: Consider first the case of a finite set $I = \{1, 2, \ldots, n\}$. Let $x_0 \in X$ and let U be an open set in Y such that $f(x_0) = \prod_{i=1}^{n} f_i(x_0) \subset U$. By a theorem of A. Weil, the compact space Y_i can be regarded as a uniform space.[5] Since $f_i(x_0)$ is a compact set in the uniform space Y_i, and U is an open set in Y containing $\prod_{i=1}^{n} f_i(x_0)$, there exists for each i an open set U_i in Y_i such that $f_i(x_0) \subset U_i$ $(1 \leqq i \leqq n)$ and $\prod_{i=1}^{n} U_i \subset U$. As f_i is u. s. c., there is a neighborhood W_i of x_0 such that $f_i(x) \subset U_i$ for $x \in W_i$. Then $W = \bigcap_{i=1}^{n} W_i$ is a neighborhood of x_0 such that $f(x) = \prod_{i=1}^{n} f_i(x) \subset \prod_{i=1}^{n} U_i \subset U$ for all $x \in W$.

Next consider the case of an infinite set I. Let $x_0 \in X$ and let U be an open set in Y such that $f(x_0) = \prod_{\nu \in I} f_\nu(x_0) \subset U$.

If $f_{\nu_0}(x_0) = \phi$ for some $\nu_0 \in I$, then, as f_{ν_0} is u. s. c., we can find a neighborhood W of x_0 such that $f_{\nu_0}(x) = \phi$ for all $x \in W$. This implies that $f(x) = \prod_{\nu \in I} f_\nu(x) = \phi \subset U$ for all $x \in W$.

Assume now $f_\nu(x_0) \neq \phi$ for each $\nu \in I$. Since U is a union of elementary open sets (i.e., basic prisms) of Y and $\prod_{\nu \in I} f_\nu(x_0)$ is compact, there exists a finite number of elementary open sets $U^{(j)}$ in Y $(1 \leqq j \leqq m)$ such that

$$\prod_{\nu \in I} f_\nu(x_0) \subset \bigcup_{j=1}^{m} U^{(j)} \subset U. \tag{2}$$

These m sets $U^{(j)}$ are of the form

$$U^{(j)} = \prod_{i=1}^{n} U_{\nu_i}^{(j)} \times \prod_{\substack{\nu \in I \\ \nu \neq \nu_i}} Y_\nu,$$

where $U_{\nu_i}^{(j)}$ is open in Y_{ν_i}. As each $f_\nu(x_0) \neq \phi$, (2) implies

$$\prod_{i=1}^{n} f_{\nu_i}(x_0) \subset \bigcup_{j=1}^{m} \prod_{i=1}^{n} U_{\nu_i}^{(j)},$$

where the right-hand side is an open set in $\prod_{i=1}^{n} Y_{\nu_i}$. From what we have just proved for the case of a finite set I, the transformation $x \to \prod_{i=1}^{n} f_{\nu_i}(x)$ from X into $\mathfrak{C}\left(\prod_{i=1}^{n} Y_{\nu_i}\right)$ is u. s. c. Hence, there exists a neighborhood W of x_0 such that

$$\prod_{i=1}^{n} f_{\nu_i}(x) \subset \bigcup_{j=1}^{m} \prod_{i=1}^{n} U_{\nu_i}^{(j)} \text{ for all } x \in W.$$

This implies that

$$f(x) = \prod_{\nu \in I} f_\nu(x) \subset \bigcup_{j=1}^{m} U^{(j)} \subset U \text{ for all } x \in W.$$

THEOREM 2. *Let $\{L_\nu\}_{\nu \in I}$ be a family (finite or infinite, not necessarily countable) of locally convex topological linear spaces. For each $\nu \in I$, let K_ν be a compact convex set in L_ν and let $H_\nu = \prod_{\substack{\lambda \in I \\ \lambda \neq \nu}} K_\lambda$. Let $K = \prod_{\nu \in I} K_\nu$ and let $\{E_\nu\}_{\nu \in I}$ be a family of closed subsets of K. If, for any point $x \in K$ and for any $\nu \in I$, the set in K_ν*

$$pr_{K_\nu}[E_\nu \cap (K_\nu \times pr_{H_\nu} x)] \tag{3}$$

is non-empty and convex, then $\bigcap_{\nu \in I} E_\nu \neq \phi$.

Proof: As $K = K_\nu \times H_\nu$, the set (3) is the section of E_ν determined by the point $pr_{H_\nu} x$ of H_ν, and therefore may be denoted by $E_\nu(pr_{H_\nu} x)$. By Lemma 2, the transformation $pr_{H_\nu} x \to E_\nu(pr_{H_\nu} x)$ from H_ν into $\mathfrak{C}(K_\nu)$ is u. s. c. On the other hand, the projection from K onto H_ν is a continuous point-to-point transformation. Hence the transformation $x \to E_\nu(pr_{H_\nu} x)$ from K into $\mathfrak{C}(K_\nu)$ is u. s. c. and therefore, by Lemma 3, the transformation f from K into $\mathfrak{C}(K)$ defined by $f(x) = \prod_{\nu \in I} E_\nu(pr_{H_\nu} x)$ is u. s. c. Furthermore, as $E_\nu(pr_{H_\nu} x)$ is non-empty and convex, we have $f(x) \in \mathfrak{K}(K)$. By Theorem 1, there exists a point $x \in K$ such that $x \in f(x)$, which means $x \in \bigcap_{\nu \in I} E_\nu$.

Using the case $I = \{1, 2\}$ of Theorem 2, one can easily prove:

THEOREM 3. *Let L_1, L_2 be two locally convex topological linear spaces, and K_1, K_2 be two compact convex sets in L_1, L_2, respectively. Let f be a real-valued continuous function on $K_1 \times K_2$. If, for any $x_0 \in K_1$, $y_0 \in K_2$, the sets $\{x \in K_1 \mid f(x, y_0) = \operatorname{Max}_{\xi \in K_1} f(\xi, y_0)\}$ and $\{y \in K_2 \mid f(x_0, y) = \operatorname{Min}_{\eta \in K_2} f(x_0, \eta)\}$ are convex, then*

$$\operatorname{Max}_{x \in K_1} \operatorname{Min}_{y \in K_2} f(x, y) = \operatorname{Min}_{y \in K_2} \operatorname{Max}_{x \in K_1} f(x, y).$$

Theorem 3 includes Ville's minimax theorem, which we state in a much more general form: *Let X_1, X_2 be two compact spaces and let φ be a real-valued continuous function on $X_1 \times X_2$. Let M_i^* $(i = 1, 2)$ be the set of all regular Borel measures on X_i with total measure* 1. *Then*[10]

$$\operatorname*{Max}_{\mu_1 \in M_1^*} \operatorname*{Min}_{\mu_2 \in M_2^*} \int_{X_1 \times X_2} \varphi d(\mu_1 \times \mu_2) = \operatorname*{Min}_{\mu_2 \in M_2^*} \operatorname*{Max}_{\mu_1 \in M_1^*} \int_{X_1 \times X_2} \varphi d(\mu_1 \times \mu_2).$$

We also mention that a direct application of Theorem 2 yields the following result on systems of linear equations with dominant diagonal coefficients: *Let* $\sum_{\nu \in I} a_{\lambda\nu} x_\nu = b_\lambda (\lambda \in I)$ *be a system (finite or infinite, not necessarily countable) of linear equations with real coefficients such that for each fixed* $\lambda \in I$, *the family* $\{a_{\lambda\nu}\}_{\nu \in I}$ *is summable.*[11] *If* $a_{\lambda\lambda} \geqq \sum_{\substack{\nu \in I \\ \nu \neq \lambda}} |a_{\lambda\nu}| + |b_\lambda|$ *for each* $\lambda \in I$, *then the system has a solution* $\{x\}_{\nu \in I}$ *such that* $-1 \leqq x_\nu \leqq 1$ *for each* $\nu \in I$.

* This work was supported in part by the Office of Naval Research.

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[8] For topological terms and notations, we agree with Bourbaki, N., *Topologie Générale*, Chap. I–II, Hermann, Paris, 1940. Especially, for the theory of uniform spaces, see Chap. II of this book.

[9] A *topological linear space* is a real vector space topologized with a separated topology such that the vector operations are continuous. A topological linear space L is *locally convex*, if the null-element of L has a fundamental system (not necessarily countable) of neighborhoods formed exclusively by convex sets.

[10] Let C_i^* $(i = 1, 2)$ be the conjugate space of the Banach space C_i of all real-valued continuous functions on X_i. Then, with respect to the w^*-topology (i.e., the weak topology of C_i^* induced from C_i), C_i^* is a locally convex topological linear space, in which M_i^* is a compact convex set. Moreover $\int_{X_1 \times X_2} \varphi d(\mu_1 \times \mu_2)$ is a continuous function on $M_1^* \times M_2^*$ (with respect to the w^*-topology).

[11] A family $\{a_\nu\}_{\nu \in I}$ of real numbers is *summable* and has *sum* s, if, for any $\epsilon > 0$, there exists a finite subset J_0 of I such that $|s - \sum_{\nu \in J} a_\nu| < \epsilon$ for every finite subset $J \supset J_0$ of I. The sum s is denoted by $\sum_{\nu \in I} a_\nu$.

ON A CONVENIENT SYSTEM OF PARAMETERS FOR THE UNITARY GROUP

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The 2-dimensional unitary group is a 4-parameter group and two of these parameters (the class parameters) may be taken to be the two angles α_1 and α_2 of a typical element of the group. Every 2×2 unitary matrix U_2 may be factored as follows

$$U_2 = VAV^{-1}$$

where

$$A = \begin{pmatrix} e^{i\alpha_1} & 0 \\ 0 & e^{i\alpha_2} \end{pmatrix} \text{ and } V = \begin{pmatrix} \cos\theta e^{i\tau} & -\sin\theta \\ \sin\theta & \cos\theta e^{-i\tau} \end{pmatrix}.$$

Here θ is a quarter-turn angle, which ranges over the interval $[0, \pi/2]$; τ is a whole-turn angle, which ranges over the interval $[-\pi, \pi]$, and the four angles α_1, α_2, θ, τ form a convenient system of parameters for the 2-dimensional unitary group, α_1 and α_2 (which are whole-turn angles) being the class parameters and (θ, τ) being the in-class parameters. The element of volume dV_2 of the 2-dimensional unitary group, in terms of these parameters, is the product of two factors one of which, the class factor, involves only the class parameters while the other, the in-class factor, involves only the in-class parameters. The class factor, which is well known, is $\{1 - \cos(\alpha_1 - \alpha_2)\}\, d\alpha_1\, d\alpha_2$ and the in-class factor is $\sin 2\theta\, d\theta\, d\tau$.

The 3-dimensional unitary group is a 9-parameter group and three of these parameters, the class parameters, may be taken to be the three angles α_1, α_2, α_3 of a typical element of the group. We denote by $U_{12}(\theta, \tau)$ the matrix

$$\begin{pmatrix} \cos\theta\, e^{i\tau} & -\sin\theta & 0 \\ \sin\theta & \cos\theta\, e^{-i\tau} & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

and by $U_{23}(\theta, \tau)$ the matrix

$$\begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\theta\, e^{i\tau} & -\sin\theta \\ 0 & \sin\theta & \cos\theta\, e^{-i\tau} \end{pmatrix}.$$

Then any 3×3 unitary matrix U_3 may be factored as follows:

$$U_3 = VAV^{-1}$$

where

$$A = \begin{pmatrix} e^{i\alpha_1} & 0 & 0 \\ 0 & e^{i\alpha_2} & 0 \\ 0 & 0 & e^{i\alpha_3} \end{pmatrix}$$

and

$$V = U_{12}(\theta_3, \tau_3) U_{23}(\theta_2, \tau_2) U_{12}(\theta_1, \tau_1).$$

The six angles $(\theta_1, \theta_2, \theta_3, \tau_1, \tau_2, \tau_3)$, of which the θ's are quarter-turn and the τ's whole-turn angles, may be taken as the in-class parameters of the group. The class factor of the element of volume dV_3 of the group is well known to be

$$\{1 - \cos(\alpha_1 - \alpha_2)\}\{1 - \cos(\alpha_1 - \alpha_3)\}\{1 - \cos(\alpha_2 - \alpha_3)\}\, d\alpha_1\, d\alpha_2\, d\alpha_3$$

and the in-class factor of dV_3 is the product of

$$\sin 2\theta_1 \sin 2\theta_2 \sin 2\theta_3\, d\theta_1\, d\theta_2\, d\theta_3\, d\tau_1\, d\tau_2\, d\tau_3 \text{ by } \sin^2 \theta_2.$$

The 4-dimensional unitary group is a 16-parameter group and four of these parameters, the class parameters, may be taken to be the four angles $\alpha_1, \alpha_2, \alpha_3, \alpha_4$ of the typical element of the group. Any 4×4 unitary matrix U_4 may be factored as follows:

$$U_4 = VAV^{-1}$$

where

$$A = \begin{pmatrix} e^{i\alpha_1} & 0 & 0 & 0 \\ 0 & e^{i\alpha_2} & 0 & 0 \\ 0 & 0 & e^{i\alpha_3} & 0 \\ 0 & 0 & 0 & e^{i\alpha_4} \end{pmatrix}$$

and

$$V = U_{12}(\theta_6, \tau_6) U_{23}(\theta_5, \tau_5) U_{34}(\theta_4, \tau_4) U_{12}(\theta_3, \tau_3) U_{23}(\theta_2, \tau_2) U_{12}(\theta_1, \tau_1).$$

The 12 angles $(\theta_1, \ldots \theta_6, \tau_1, \ldots, \tau_6)$ (of which the θ's are quarter-turn and the τ's whole-turn angles) may be taken as the in-class parameters of the group. The class factor of the element of volume dV_4 of the group is well known to be

$$\left[\prod_{p<q} \{1 - \cos(\alpha_p - \alpha_q)\} \right] d\alpha_1\, d\alpha_2\, d\alpha_3\, d\alpha_4.$$

The in-class factor is the product of

$$\sin 2\theta_1 \sin 2\theta_2 \ldots \sin 2\theta_6\, d\theta_1 \ldots d\theta_6\, d\tau_1 \ldots d\tau_6 \text{ by } (\sin^2 \theta_2)(\sin^4 \theta_4 \sin^2 \theta_5).$$

In general the n-dimensional unitary group is an n^2-parameter group. The n angles $(\alpha_1, \ldots, \alpha_n)$ of a typical element of the group may be taken

as n of the n^2 parameters and these are known as the class parameters (the remaining $n^2 - n$ being in-class parameters). Any $n \times n$ unitary matrix U_n may be factored as follows:

$$U_n = VAV^{-1}$$

where

$$A = \begin{pmatrix} e^{i\alpha_1} & 0 & 0 & \cdot & \cdot & \cdot & 0 \\ 0 & e^{i\alpha_2} & 0 & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ 0 & 0 & \cdot & \cdot & \cdot & \cdot & e^{i\alpha_n} \end{pmatrix}$$

and

$$V = U_{12}(\theta_{n(n-1)/2}, \tau_{n(n-1)/2}) U_{23}(\ ,\ ) \ldots U_{n-1n}(\ ,\ ) \\ U_{12}(\ ,\ ) \ldots U_{12}(\theta_1, \tau_1).$$

The $n(n-1)$ angles θ and τ (of which the θ's are quarter-turn and the τ's whole-turn angles) may be taken as the in-class parameters of the group. The class factor of the element of volume dV_n of the group is well known to be

$$\left[\prod_{p<q} \{1 - \cos(\alpha_p - \alpha_q)\} \right] d\alpha_1 \ldots d\alpha_n$$

and the in-class factor of dV_n is the product of

$$\sin 2\theta_1 \sin 2\theta_2 \ldots \sin 2\theta_n \, d\theta_1 \ldots d\theta_n \, d\tau_1 \ldots d\tau_n$$ by $$(\sin^2\theta_2)(\sin^4\theta_4 \sin^2\theta_5) \\ (\sin^6\theta_7 \sin^4\theta_8 \sin^2\theta_9) \ldots .$$

In this latter product there are $n - 2$ parentheses of which the the last is $(\sin^{2n-4}\theta_{(n^2-3n+4)/2} \ldots \sin^2\theta_{(n-2)(n+1)/2})$.

ON THE MODULAR REPRESENTATIONS OF THE SYMMETRIC GROUP (II)

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1. In his Thesis J. H. Chung[2] put forward two conjectures, the first[6, 7] to the effect that the number of ordinary (modular) irreducible representations of the symmetric group S_n belonging to a p-block of given weight is independent of the p-core, and the second that an indecomposable of the regular representation of S_n is characterized by the *linkage* property holding for all removable kp-hooks, for every k.

D. E. Littlewood[3] has recently suggested an alternative approach to the subject. Construct an infinite matrix $A = (a_{ij})$ where:

(i) $a_{ij} \equiv j - i + 1 \pmod{p}$, $\quad (i, j = 1, 2, \ldots)$
(ii) $0 < a_{ij} \leq p$;

then Littlewood's *p-graph* of a Young diagram $[\alpha]$ is the part of A overlapped by $[\alpha]$, when the first row and column of $[\alpha]$ are made to coincide with the first row and column of A. The integer a_{ij} associated thus with the (i, j) node of $[\alpha]$ determines the *p-class* of that node. Clearly an arbitrary constant could be added to every a_{ij}, for example -1, without affecting the significance of the idea. We make the following observations:

(1) If $a_{ij} = r$, then $a_{i, j-1} = a_{i+1, j} \equiv r - 1 \pmod{p}$.

(2) Thus any skew p-hook with head node[8] of class r has a p-graph containing a sequence of integers congruent (mod p) to:

$$r, r - 1, r - 2, \ldots, r - (p - 1) \equiv r + 1 \pmod{p},$$

reading from head to foot.

(3) An analogous statement holds for any skew kp-hook.

We conclude that the class of its head node can thus be associated with a skew kp-hook, though, of course, this is independent of the leg length of the hook. It follows that a necessary and sufficient condition that $[\alpha]$ be a p-core is expressible in terms of the classes of nodes appearing on the rim of the diagram.[9]

Moreover, all the p-hooks represented by the nodes of a disjoint constituent of the star diagram will be associated with the same class, so that we have an alternative proof that the number of such constituents is at most p. This associated class is preferable to the leg length of the upper left-hand corner node's corresponding p-hook, in that it is associated with every node of the constituent. The proof of the theorem that $[\alpha]$ exists and is completely determined by its p-core and star diagram, with associated residue classes,[7] carries through on this new basis.

2. In his discussion of the indecomposables of the regular representation, Littlewood thinks of adding in succession the integers 1 to p, with repetitions allowed, to build the p-graph of $[\alpha]$. He states that, corresponding to each indecomposable there exists a permutation S of $(1^a 2^b \ldots p^\epsilon)$ which is *admitted* by the p-graphs of its irreducible components, but makes no comment on the fact that this permutation is not uniquely defined. It was just this ambiguity which aroused the present author's interest, since it would be desirable that an explicit characterization of an indecomposable should simultaneously characterize the corresponding modular irreducible representation.

To obtain such a characterization one must write down all possible permutations S admitted by the irreducible components of an indecompos-

able, realizing that a given permutation may be admitted in more than one way. Counting all such mutiplicities, the number of such permutations is just equal to the degree of the modular irreducible representation in question. For example (see also §3), the 3-graphs of the representations [4, 1] and [3, 2] which are the irreducible components of an indecomposable representation of S_5 admit the permutations:

12313; 12331, 12331, 13231.

These permutations indicate that if the corresponding modular irreducible representation of S_5 be restricted to S_4 then it will split into the identity representation [4] which admits the permutation 1231, and the modular irreducible representation [3,1] which admits the permutations: 1233, 1233, 1323. We may denote the process involved by 3-restricting, or 1-restricting or in general by *s-restricting*.

The inducing process, which is inherent in any general approach to these ideas; consists of adding a node in all possible ways.[5] Chung saw that applying this process to an indecomposable, Nakayama's formulae (reference 1, p. 582 (83)), though requiring the result to be a sum of indecomposables, did not provide a means of separating them out. It was this difficulty which led to the formulation of his "minimum complete linkage" hypothesis. Now it can be shown that *Chung's hypothesis is equivalent to the adding of a node of some class r in all possible ways to the p-graphs of a given indecomposable;* we shall call this *r-inducing*.

If these constructions be taken to define the modular irreducible representations and the indecomposables, then *r*-inducing and *s*-restricting certainly satisfy the reciprocity requirements of Nakayama's formulae. It is to be noted that they are not applicable to the alternative formulae (*loc. cit.* (85)).

Littlewood's "*consecutive application of the numbers of the permutation S*" (reference 5, p. 348) amounts to successive *r*-inducing and so to Chung's hypothesis. But Littlewood is not clear regarding his procedure when faced with the overlapping of two indecomposables.

It does not seem possible to decide the question at this stage, but there is no *a priori* reason why the irreducible constituents of two indecomposables should not thus overlap. Chung showed that his hypothesis provided a unique determination of the indecomposables for $n = 2p$, and in consequence so also does the more explicit procedure of *r*-inducing. Such a characterization of an indecomposable is so elegant that one can at least hope that it proves to be correct in the general case.

3. For $n = p$, these ideas can be illustrated by means of a familiar formula connecting binomial coefficients:

$$\binom{p-1}{0}^2 + \binom{p-1}{1}^2 + \ldots + \binom{p-1}{p-1}^2$$
$$= \binom{p-2}{0}\binom{p}{1} + \binom{p-2}{1}\binom{p}{2} + \ldots + \binom{p-2}{p-2}\binom{p}{p-1}.$$

The terms on the left represent the squares of the degrees

$$\binom{p-1}{x}$$

of the hook representations $[p-x, 1^x]$ of S_p, while the relation

$$\binom{p}{x} = \binom{p-1}{x} + \binom{p-1}{x-1}$$

implies that the p-binomial coefficients on the right are the degrees of the indecomposables of weight 1. The $(p-2)$-binomial coefficients are the degrees of the corresponding modular irreducible representations,[1] and these fit together as shown by Nakayama.[4]

It is perhaps worth giving in conclusion the five classes of permutations admitted by the 3-graph of [3, 2, 1]:

A: 123123
B: 123132, 123312, 123312, 132312
C: 123321, 123321, 132321, 132231, 132231, 123231
$\bar{B}$: 132123, 132213, 132213, 123213
$\bar{A}$: 132132

The corresponding modular representations are related to the ordinary representations of S_6 according to the accompanying table of decomposition numbers. This table conforms, of course, to Chung's determination[2] or $n = 2p$.

	A	B	C	$\bar{B}$	$\bar{A}$
[6]	1				
[5,1]	1	1			
$[4,1^2]$		1	1		
$[3^2]$		1			1
[3, 2, 1]	1	1	1	1	1
$[3,1^3]$			1	1	
$[2^3]$	1			1	
$[2,1^4]$				1	1
$[1^6]$					1

Added in Proof (January 31, 1952). The definition of the matrix A above can be generalized by writing $a_{ij} = j - i + 1$, and the significance of *admissibility* becomes clear. This generalization will be the subject of a subsequent note.

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*THE PLACE PRINCIPLE IN AUDITORY THEORY** †

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All modern forms of auditory theory accept the place principle as operating at least in a limited way in the physiological transformation of sounds for the purposes of perception. This is the principle that every tone has its own characteristic place or pattern of action in the cochlea and hence its own particular representation by fibers in the auditory nerve. We are here concerned with the physical process by which this spatial differentiation of action in the cochlea is achieved.

Now competing in this theoretical field are two general conceptions as to the nature of this physical process. These are the resonance theories, or, more broadly, the local action theories, and the traveling wave theories. The resonance or local action theories have the advantage of age and long acceptance, and especially the authority of Helmholtz. The traveling wave theories are venerable too as judged from date of origin but were never well accepted in classical times; they gain their present advantage from a recent upsurge of interest that has led to many new formulations, and from strong claims of support in the empirical observations of Békésy. Our interest here is in making a choice between these two classes of theory.

A choice is made possible by the widely different assumptions set forth by these two classes of theory regarding the propagation of sounds through the cochlea and the manner of distribution of sensory activity within this

structure. We shall discuss these assumptions in general, in forms typical of the two classes, but without any treatment of individual theories at this time. Detailed descriptions of the theories are available elsewhere,[1] and the interested reader can easily see the specific applications.

A traveling wave theory supposes that sound vibrations communicated to the oval window by the middle ear first set up a wave of displacement in the basal portion of the basilar membrane. This wave moves progressively toward the apical end of the cochlea until it finally disappears. The action is much like that caused in a moderately slack, horizontally held rope when a sudden up-and-down movement is made at one end. Successive vibrations of course produce a continuous series of traveling waves.

As they travel the waves vary in amplitude in a manner that depends characteristically upon the vibratory frequency and upon the properties of the cochlear structures. Similarly determined is the distance that

FIGURE 1

Sound conduction in the cochlea according to the traveling wave type of theory. The two curves (*1*, *2*) represent successive views of the displacement of the basilar membrane as the wave moves up the cochlea in the direction of the arrow. This wave would be expected to expire near the middle of the membrane.

the waves go before they finally are extinguished. The theories differ in detail here, but a common pattern of this variation is one in which the wave grows in peak amplitude for a time, reaches a maximum, and then rapidly falls away, reaching zero after a few minor undulations, as illustrated in figure 1. High tones reach their maximums quickly and quickly vanish, with their actions confined to the basal region, whereas low tones proceed farther toward the apex of the cochlea. Thus a series of tones of many different frequencies will have their patterns of action spread out systematically along the basilar membrane. In some wave theories it is the point of maximum amplitude that is taken as the determiner of pitch, in others it is the point of cessation of the action, but in any event the action provides positional cues as a basis for the recognition of tones.

Because the traveling wave theories conceive of one or several waves as present on the basilar membrane in any instant, it follows that the speed of propagation along the membrane is relatively slow. The theories differ in their particular assumptions here. In some of the older theories

the speed is taken as about 1 m./sec. (meter per second). In the more recent theories the speed is regarded as varying continually as the wave progresses along the cochlea; in one theory it is said to vary from 63 to 0.05 m./sec., in another from 38 to 415 m./sec., and in a third from 45 to 3 m./sec. These speeds are all slow in relation to the speed of sound in an extended body of fluid; in sea water, for example, which probably has conductive properties much like those of cochlear fluid, this speed is 1563 m./sec. at body temperature.

For the resonance theories or other forms of local action theories there are no traveling waves; the movements set up in the basilar membrane by sounds are strictly local, not propagated along the membrane. It is true, in most forms of this theory it is agreed that a broad area of the membrane or (as we believe) the whole membrane responds to a single tone, yet each part making up this response area is directly driven by the vibrations of the cochlear fluid. Conduction through the cochlea occurs only in this fluid. The speed of this conduction is not a matter of particular concern in these theories, but it is usually regarded as high, like free conduction in fluids in general and hence of the order of 1563 m./sec.

Bearing in mind these theoretical differences between the two classes of theories, we turn to what we have designed as a crucial test situation. These experiments were carried out in deeply anesthetized cats by use of the cochlear potentials as representative of the activity of the sensory cells. The animals were prepared by opening the auditory bulla to give a wide exposure of the cochlea. An electrode in the form of a small piece of platinum foil was placed in contact with the round window membrane and another, inactive, electrode was placed in muscle tissue near by. A minute hole was drilled in the cochlea at a point near its apex, with care just to penetrate the bony wall and not to invade the soft endothelial layer that lines the inner surface of the wall. A blunt-ended steel needle attached to a crystal vibrator was oriented in this hole so as to work in it like a piston in a cylinder, with its end pressing upon the endothelial lining. When the needle was made to vibrate it set this lining and the fluid below in motion and thereby stimulated the sensory structures of the cochlea. The vibrator was driven at some chosen frequency (say, 1000 cycles per sec.) and at an intensity necessary to produce a moderate response of 10 microvolts. Also, the external auditory meatus was connected with a tube delivering aerial sounds from a loudspeaker. The loudspeaker was driven at the same frequency, through a channel operated by the same oscillator, but with independent controls of intensity and phase. This aerial stimulus was adjusted likewise to produce a response of 10 microvolts when presented by itself. Then both forms of stimulation were applied simultaneously and the phase relation between them was given various values over a range of 0 to 360°.[2]

Here we have a dual form of stimulation: (1) by a sound that enters the ear in the usual way and communicates its vibrations through the ossicular chain and oval window to the basal end of the cochlea and (2) by a sound that enters the cochlea through an artificial opening at its apical end. The effects, as we shall see, are discriminatory between the two classes of theory.

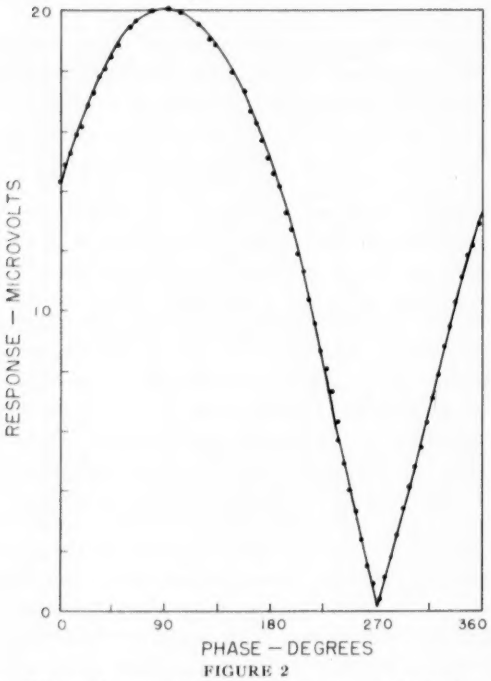

FIGURE 2

Effects of phase relation on the responses obtained to a 5000 cycle tone applied simultaneously at two cochlear positions, by way of the oval window and through a hole near the apex. The designation of phase has no absolute significane; actually there is reason to believe that the null is obtained for a 180° phase relation.

The effects are very simply described in this way: the two forms of stimulation give responses representing at all times the vectorial sum of the responses that they elicit when acting independently. Figure 2 shows some representative results. At a certain phase relation the two stimulations combine their effects to produce double the response of either; at a phase relation that is 180° removed from this one the effects combine to give a null. At this null point the balancing of the two stimuli is highly critical and any slight change in phase relation or in the intensity of either stimulus destroys the null.

This vectorial summation of the two stimulations holds for all frequencies. Tests with numerous tones over the range of 200 to 15,000 cycles gave results like those of figure 2.

For all tones of low and intermediate frequency—specifically, for tones below 500 cycles per sec.—a null once found continued to hold regardless of the location of the electrode. The principal tests were made with the

electrode on the round window membrane, but it could be located anywhere on the bony capsule of the cochlea, or at the margin of the drilled hole, and the null point continued to be found for the same phase and intensity relations as before. This fact is of great significance. It means that the summation of the two stimulations is occurring in every active cell, for otherwise a shifting of position would favor one means of stimulation over the other and upset their precarious balance. When we use one form of stimulation only and move the electrode about in this way we do indeed obtain various amounts of response depending evidently upon conditions of local conductivity: such things as distance of conduction of the potentials, thickness of the bony capsule, and moistness of its surface. The form of the summational effect that we have described here is not influenced by these superficial conditions. A change of electrode position affects the magnitude of response for both forms of stimulation equally and their vectorial relation remains unchanged.

As far as the form of the results is concerned there is nothing critical about the location of the drilled hole. It may be in the middle of the cochlea, for example, and the same kind of summation occurs. Also, the vibrating needle may be placed on the bony surface with the same result. It may be moved to any position on the surface from the apical end to the basal end, with still the same result. When for a given tone the intensity and phase relations are set for a null these settings hold for all these stimulating positions, in the drilled hole and anywhere on the bony surface, with only minor adjustments ($\pm 5°$) that are within the usual experimental variations for a single position.

These observations lead to the following conclusions. Every form of stimulation—by way of the oval window, through a hole in the cochlear capsule in any location, and by bone conduction from any point of application—produces the same pattern of effect upon the cochlea. The vibrations are conducted through the fluid to all parts of the structure at a rapid rate and with no appreciable loss of amplitude.

For high tones, those above 5000 cycles, phase changes are observed as a function of cochlear position, but the changes are always small with respect to the periods of these waves, at least up to 15,000 cycles.

We can determine the order of magnitude of the time of conduction through the cochlea from our results. This time must be small relative to the periods of all tones for which nulls have been obtained. Hence this time must be small relative to $^1/_{15,000}$ sec. = 67 microseconds. In a particular one of our experiments the hole was drilled at a point 18.5 mm. from the basal end of the cochlea, and it is estimated that in this case the mean fluid path between the two points of application of the sound was 15.8 mm. A sound traveling at a speed of 1563 m./sec. will cover this distance in approximately 10 microseconds. This time of 10 microseconds

only barely satisfies our condition of smallness relative to 67 microseconds and thus we conclude that the speed of conduction is very rapid.

The attenuation of intensity in passing through the cochlea must be small, for otherwise most of the active cells would receive unequal amounts of stimulation from the two sources, and though a balance might be found for some one electrode position near the middle of the cochlea it could not exist for any other, more remote, electrode positions. Though we have made no special effort to obtain data bearing on this point, our belief is that the attenuation from one end of the cochlea to the other cannot exceed 1 db.

These conclusions are wholly unfavorable to a traveling wave theory. This form of theory, as we have seen, assumes a slow, decremental conduction of effects along the basilar membrane. If a wave beginning at the oval window is subjected to progressive decay until soon it disappears, say, in the middle of the cochlea, so also a wave beginning at the apex must decay and disappear. Such a pattern falls under the discussion of the preceding paragraph and is precluded by our results. Whatever the theory may say about conduction through the fluids—whether it is presumed to exist or not to exist—is now irrelevant, for a wave traveling along the membrane in the manner described would prevent the balancing of effects as shown in our results. Of course, from these results we could not object to the assumption that the membrane propagates its wave through the cochlea instantaneously and without attenuation; but such an assumption defeats the purpose of a traveling wave theory.

What remains for acceptance is the resonance theory or any other form of local action theory, in which the forces of the vibratory stimulus are distributed throughout the cochlea and produce movements in portions of the basilar membrane and its sensory structures according to their local characteristics. These movements may bear any and various relations of phase, time, or intensity with respect to the acting forces, always depending upon the stimulating frequency and the mechanical characteristics of the responding structures. If such characteristics of the responding structures vary progressively along the cochlea, then the patterns of action in response to a tone will show regional and temporal variations correspondingly. Such patterns might on visual examination give the appearance of a traveling wave, and we suppose that this is the basis for Békésy's observations. This appearance is illusory only and does not represent traveling waves in fact; and here is a fundamental distinction affecting our whole conception of the manner of operation of the ear.

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THE ISOLATION AND CHEMICAL PROPERTIES OF THE NUCLEOLI OF STARFISH OOCYTES

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The function of several of the discrete protoplasmic structures has been more completely understood where the structure itself has been isolated and studied by direct biochemical methods.[1] Progress toward the understanding of the chemical nature and of the function of the nucleolus has been hindered by the lack of a suitable technique for the isolation of nucleoli in quantities suitable for direct chemical study. During an attempt to analyze the role of the nucleolus in the growth stages of the oocytes of various forms, a technique for the isolation of nucleoli in quantities suitable for biochemical analyses was developed. This technique and a preliminary report of the chemical composition of the starfish nucleolus is given below.

The isolation procedure described has been successfully applied to the oocytic nucleoli of two starfishes: *Asterias forbesii* and *A. rubens;* the surf clam, *Mactra solidissima;* and spider crabs of the genus *Maja*. The observations and analytical data given below apply exclusively to the nucleolus of *Asterias rubens*.[2]

Isolation Procedure.—1. Preparation and Homogenization of Material: 15–20 cc. of washed ovaries or exuded eggs in the germinal vesicle stage are allowed to stand for 20 minutes in 100 ml. of water. The tissue is then squeezed through 4 layers of cheesecloth to remove connective tissue and other debris. The cheesecloth is then washed with 100 ml. of water. The combined washing and filtrate are homogenized by being forced through a No. 18-gauge hypodermic needle with a large syringe. The homogenate is filtered through 8 to 12 layers of cheesecloth, and the cheesecloth washed with 100 ml. of water.

During the development of the technique, it was found that the homogenate generally maintained a pH of 6.0 ± 0.15 without the addition of other buffering agents. This was found to be the optimum pH for isolation, as considerable agglutination of the cytoplasm took place at levels not far above or below pH 6, particularly in the presence of salts. For this reason distilled water was found to be the most suitable isolation medium. Pyrex redistilled water was used throughout, and all operations were carried out in the cold room at 2–4°.

2. Sedimentation of Nucleoli: The filtered homogenate is placed in 50-ml. centrifuge cups and centrifuged for 20 minutes at 375 g. The supernate is discarded and the surface of the pellet washed with a few milliliters

of water. The pellet is then resuspended in 20 volumes of water and centrifuged for 15 minutes at 375 g. This procedure is repeated twice, or until the proteinaceous gel is removed. The remainder of the purification is carried out in a hand centrifuge. As the time of centrifuging is quite short, the centrifuging periods are referred to in terms of the number of turns of the handle required.

The centrifuge used during the development of the technique handled 15-ml. conical centrifuge tubes and had a 14 cm. radius to the bottom of the tubes with a 15:1 gear ratio. The handle was consistently turned at the rate of two revolutions per second.

3. Removal of Large Cell Debris and Membranes: The centrifugate is resuspended in 10 ml. of water and transferred to a 15-ml. conical centrifuge tube. It is then centrifuged 10 turns in the hand centrifuge. The supernate is filtered through one or two layers of bolting silk (50 × 50 micron mesh) and the centrifugate discarded. This step is repeated until microscopic examination of the supernate shows a minimum of nuclear and membrane contamination. (The filtration of the nucleoli should be kept to a minimum, as large numbers of nucleoli adhere to the fabric and are lost.) Finally, the nucleolar suspension is concentrated by 100 turns in the hand centrifuge.

4. Removal of Small Particles and Somatic Nuclei: The concentrate is suspended in 10 ml. of water, centrifuged 60 turns and the supernate discarded. This step is repeated 2 to 4 times, or until microscopic examination reveals no nuclei.

5. Control of Preparation Purity: The following steps were taken to insure maximum purity in the final preparation. Each step of the isolation was controlled by examining a small quantity of the preparation under the microscope. A small quantity of 0.01% toluidine blue was added to the suspension on the slide to aid in identifying the nucleoli. The final preparations were examined with toluidine blue and with the phase microscope. To detect nuclear and cytoplasmic contamination the preparations were also stained with pyronin-methyl green. The low centrifugal forces used in the isolation practically precluded contamination of the preparations by the granules of the cytoplasm, and no such contamination was detected in any preparation. In cases where shed eggs were used as a source of nucleoli, the egg membranes were found to be a major contaminant. At the suggestion of Dr. Seymour Cohen, the following technique to remove this contamination was adapted from a technique for purifying virus described by Topping.[3] After step 2, the centrifugate is suspended in 1 volume of water and $1^1/_2$ volumes of ether, shaken thoroughly and centrifuged for 15 minutes at 375 g. The protein material comes to lie at the ether-water interface, trapping the membranes and other large particles with it, while the nucleoli are concentrated at the bottom of the bottle. The nucleoli

are removed with a fine pipette and transferred to a 15-ml. centrifuge tube for step 3. This procedure will also remove microsomes and other cytoplasmic granules from the preparations. The major sources of contamination found in the final preparations were nuclei which were not disrupted by the homogenization procedure and egg membrane material. The estimated contamination in no case exceeded 5% and generally was less than 1%.

6. *Yield:* The yield from a typical preparation was usually 5 to 10 mg. (dry weight) and 30 to 70% of the total nucleoli.

Physical Properties of the Nucleolus.—The nucleolus of the starfish oocyte is a spherical, highly refractile body containing one to many vacuoles of varying sizes.

The nucleoli demonstrate a definite change in appearance upon contact with the isolation medium. This change is simliar to that seen in nuclei which are isolated by the citric acid technique. The change appears as an increase in the refractive index of the nucleolus. Upon prolonged contact with distilled water, the surface of the nucleolus changes from a clear, hyaline state to a granular, less refractile condition. This change becomes accentuated in the presence of calcium binding agents such as oxalate or citrate. No other change in the size or internal structure of the nucleolus has been observed during the isolation procedure. The nucleolus does not flatten appreciably under pressure, but rather fragments into unequal pieces when excess pressure is applied. There is no evidence that it possesses an osmotic membrane. The nucleolus swells very greatly in alkaline media. This swelling begins at about pH 7.6, and is reversible with the addition of acid. There is no evidence of structural change (such as alteration in size or number of vacuoles, etc.), even after two or three alternate exposures to alkali and acid. The normal size of the nucleolus is little affected by acid conditions. The nucleolus is rapidly broken down by dilute solutions of trypsin. It does not dissolve in salt solutions or strong acid, and dissolves slowly in *N* NaOH. The density of isolated nucleoli, as measured in aqueous sucrose solutions, is between 1.35 and 1.4.

From this evidence and visual observation, it is concluded that the nucleolus, at least after isolation, is a solid or semisolid structure.

Chemical Properties of the Nucleolus.—1. Nucleic Acid: The nucleic acid of the nucleolus is entirely of the ribose type. No desoxyribose or thymine could be detected by colorimetric or chromatographic means. The content of ribonucleic acid (RNA) in five different nucleolar preparations ranged between 2.2 and 4.6% of the dry weight of the nucleolus. A series of determinations was made on the relative base content of the RNA of the nucleolus and on the RNA of the cytoplasmic granules, using the chromatographic technique of Markham and Smith.[4] A marked difference in the ratio of guanine and uracil between the two nucleic acids was

found. The results on a molar ratio basis are given below (the mean of all the molar values = 1).

MOLAR RATIOS

BASE	NUCLEOLUS	CYTOPLASM
Guanine	1.45 ± 0.021	1.23 ± 0.016
Adenine	0.87 ± 0.015	0.80 ± 0.020
Cytosine	1.09 ± 0.014	1.15 ± 0.012
Uracil	0.59 ± 0.025	0.88 ± 0.014

Seven determinations were made on each base on material taken from three different nucleolar preparations. The deviations listed are the standard errors. The differences between the amounts of adenine and cytosine in the two nucleic acids are probably not significant (t test).

2. *Nucleolar Protein:* The nucleolus (dry weight) contains 16 to 20% nitrogen. Acid extraction removes 25 to 35% of this amount. The nature of the protein has been examined by solubility and by chromatographic means. Using the technique for extraction of basic proteins described by Daly, Mirsky, and Ris,[5] no histone could be found in the intact nucleolus. Some material with the characteristics of protamines was extracted at 60°. This extraction did not alter the size or appearance of the nucleolus. The extracted residue was hydrolyzed in 6 N HCl for 24 hours at 105° in a sealed tube, and the hydrolysate chromatographed in butanol-ammonia to separate the amino acids. The following amino acids were identified: glutamic acid, proline, arginine, lysine, glycine, tyrosine, phenylalanine, valine, leucine and isoleucine. The presence of tryptophan was established by a positive Hopkins-Cole reaction. Histidine could not be detected on the chromatogram. The amounts of glutamic acid, proline, arginine, lysine, histidine and phenylalanine differed markedly from that found in thymus histone. This protein residue, which makes up the bulk as well as the structural framework of the nucleolus, contains 0.5 to 1% phosphorus.

3. *Nucleolar Enzymes:* Tests were made to determine the presence of certain enzymes in the nucleolus. The results are tabulated below for frozen-dried nucleolar preparations and for a similar quantity of identically treated whole egg powder.

ENZYMATIC ACTIVITY

ENZYME	NUCLEOLUS	EGG POWDER
DPN reductase	−	+
Dipeptidase (alanylglycine)	−	+
Alkaline glycerophosphatase	−	+
Acid glycerophosphatase	+	+

The egg powder was also able to reduce methylene blue, both in the ab-

sence and the presence of succinate. The nucleolus did not reduce methylene blue under these circumstances.

The techniques used were sensitive to at least one-tenth of the activity found in the whole egg material, so that any significant concentration of enzyme in the nucleolus would have been detected. The activity of nucleolar acid phosphatase was approximately one-fifth that of the whole egg powder on a dry weight basis. On a nitrogen basis, the activity would be less than one-tenth that of the whole egg material.

4. Other Substances: A small amount of material which contained about 5% of the total phosphorus of the nucleolus was extracted by alcohol-ether at 60°. This treatment did not alter the appearance of the nucleolus. Also a small amount of material was found which could be extracted in cold dilute acid. This material, from absorption and chromatographic data, appeared to be a nucleotide or mixture of nucleotides, but has not yet been positively identified.

Discussion.—From these data, it is evident that the structural basis of the nucleolus of the starfish is phosphoprotein. This protein is present in high concentrations, and constitutes the bulk of the nucleolus. The failure to find significant amounts of basic protein is not in accord with Caspersson's concept that the nucleolus consists primarily of protein rich in basic amino acids.[6] The failure to find histone in the starfish nucleolus agrees with the results of Pollister and Ris, who failed to find histone in the nucleolus of maize using microspectrophotometric techniques.[7]

Subsequent to the studies of Caspersson and Schultz,[8] and Brachet,[9] the nucleic acid of the nucleolus has been considered to be of the ribose type. The chromatographic analyses made during the present study have confirmed this view. The concentration of nucleotides in the starfish nucleolus cannot be considered as being very high. The granular fraction of the starfish cytoplasm (containing some mitochondria and the microsomes) contained approximately 10% RNA as compared with the value of 3 to 4% for the nucleolus. The only other value for the nucleic acid content of the nucleolus which could be found in the literature is that given by Caspersson and Schultz[8] for the nucleolus of the salivary gland of a female Drosophila. They estimated that the absorption curve which they obtained microspectrophotometrically could be approximated by a nucleoprotein solution containing 3.5% nucleic acid and a protein of the globulin type. The male Drosophila salivary gland nucleolus was said to have an absorption curve with about twice this value of nucleic acid, but with another type of protein. In the same paper, these authors gave absorption curves for sea urchin oocyte nucleoli and spinach nucleoli. They stated that the nucleotide concentration was much higher in these forms, but no values were given.

The demonstration that the nucleolus contains a different RNA from that

of the cytoplasmic granules complements the work of Marshak,[10] who found that comparable differences existed between the nuclear and cytoplasmic RNA of several tissues. In contrast to the results of Marshak, no significant difference with respect to adenine was found between the nucleolus and cytoplasm of *Asterias rubens*.

Except for the failure to find alkaline phosphatase, the lack of enzymatic activity of the nucleolus is not surprising, in view of the low level of enzymatic activity of the nucleus as a whole. In the case of alkaline phosphatase, the histochemical technique for *in situ* localization of this enzyme on tissue sections has generally shown the nucleolus to be the site of intense activity. It is possible that the enzyme may have been lost in the aqueous media during the course of isolation, as has been found in the case of nuclei.[11] However, the detailed study of Cleland[12] on the mechanism of the histochemical demonstration of alkaline phosphatase has shown that considerable caution must be exercised in interpreting the distribution patterns of the enzyme, particularly in the nucleus. He has found that alkaline phosphatase is localized in the large granule, and in the "soluble" fractions of cell homogenates depending upon the organ, with little or no activity in the nucleus. The results of the present study support this contention.

The weak activity of acid phosphatase in the nucleolus may be due to contamination or adsorption from the cytoplasm. Such contamination in isolated nuclei has been found by Pallade.[13] The very high activity of the enzyme in the whole egg material would make such an artifact quite possible.

Summary.—A technique for the isolation of nucleoli from the oocytes of marine eggs has been described.

The nucleolus of *Asterias rubens* has been shown to be composed largely of phosphoprotein, which makes up its main structural component. Associated with the protein component is a significant amount of RNA, small amounts of lipid and soluble nucleotide.

No histone could be demonstrated in the nucleolus.

The ribonucleic acid of the nucleolus has been shown to differ from that of the cytoplasm in its content of guanine and uracil.

The only enzymatic activity detected in the nucleolus was that of acid phosphatase.

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POTENTIAL THEORY IN SPACE OF n DIMENSIONS (PART I)

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1. In order to develop Potential Theory in a Euclidean space of n dimensions, the following extension of Newton's Law of Gravity is adopted.

Newton's Law of Universal Gravitation. In a Euclidean Universe of n dimensions, any two particles of masses m_1, and m_2 situated at the positions P_1 and P_2 attract each other with a force directed along the line joining P_1 and P_2. The magnitude of the force F is directly proportional to the product of the masses and inversely proportional to the $(n-1)$ power of the distance r between P_1 and P_2. Thus

$$|F| = \frac{km_1m_2}{r^{n-1}}. \tag{1}$$

For $n = 3$, this reduces to Newton's well-known inverse square law, and for $n = 2$, this becomes the law of gravity used in the theory of the Logarithmic Potential. Thus by our work, both theories can be developed from a uniform point of view.

2. Let $x = x^i = (x^1, \ldots, x^n)$ denote cartesian coordinates in a Euclidean space of n dimensions so that the square of the differential of arc length is $ds^2 = \delta_{ij}\,dx^i\,dx^j$, where the δ_{ij} are the Kronecker deltas. An N dimensional hypersurface or Riemannian manifold V_N where $1 \leqq N \leqq n$, immersed in this Euclidean space is the locus of points defined by the system of n equations

$$x^i = \xi^i(u) = \xi^i(u^1, \ldots, u^N), \tag{2}$$

where the $\xi^i(u)$ are real single-valued continuous functions with continuous partial derivatives of the first order over a certain region of the parametric u-space such that the rank of the jacobian matrix $(\partial x^i/\partial u^j)$ is N in this region.

For this V_N, the square of the differential of arc length is given by the positive definite quadratic differential form

$$ds^2 = g_{ij}\,du^i\,du^j, \text{ where } g_{ij} = g_{ji} = \sum_{\alpha=1}^{n} \frac{\partial x^\alpha}{\partial u^i}\frac{\partial x^\alpha}{\partial u^j}. \tag{3}$$

If the determinant of the g_{ij} is $g = |g_{ij}| > 0$, the differential dV_N of hypervolume is

$$dV_N = g^{1/2}\,du^1 \ldots du^N. \tag{4}$$

A Riemannian manifold V_N together with a density function μ which is continuous over V_N, is said to constitute a material hypervolume or hypersurface. The total mass M of a material hypersurface V_N is

$$M = \int_{V_N} \cdots \int \mu\,dV_N = \int_{V_N} \cdots \int \mu g^{1/2}\,du^1 \ldots du^N. \tag{5}$$

The rectangular components of the force of attraction on a particle P situated at a position x^i and of mass m exerted by a material hypersurface $V_N: x^i = \xi^i(u)$ with the density function $\mu(u)$ are

$$X_i = km \int_{V_N} \cdots \int \frac{\mu(\xi^i - x^i)}{r^n}\,dV_N, \tag{6}$$

where r is the distance between any variable point $\xi^i(u)$ in V_N and the given point x^i.

This is found by use of an amplified form of Newton's Law of Universal Gravitation.

3. If the particle P varies over a simply connected region which does not contain the body V_N and any other masses, then the field of force is conservative. Let $U(x)$ denote a potential function such that its gradient is the force vector (6), that is, $X_i = \dfrac{\partial U}{\partial x^i}$.

If $n \geqq 3$, the potential is

$$U(x) = \frac{km}{n-2} \int_{V_N} \cdots \int \frac{\mu}{r^{n-2}}\,dV_N. \tag{7}$$

When $n = 2$, the logarithmic potentials for a wire C with arc length s and for a lamina with area S are respectively

$$U(x) = km \int_C \mu \log \frac{1}{r}\, ds, \qquad U(x) = km \iint_S \mu \log \frac{1}{r}\, dS. \quad (8)$$

4. In the given simply connected region, the field of force (6) is solenoidal since the divergence $\dfrac{\partial X_i}{\partial x^i} = 0$.

The potential function $U(x)$ of (7) and (8) is a harmonic function. Thus it obeys the Laplace equation

$$\nabla^2 U = \delta^{ij} \frac{\partial^2 U}{\partial x^i \partial x^j} = 0. \quad (9)$$

Therefore $U(x)$ is analytic over the given region.

5. In the Euclidean universe of n dimensions, let V denote the n dimensional hypervolume bounded by the hypersphere: $\delta_{ij}(x^i - x_0{}^i)(x^j - x_0{}^j) = r^2$, with center at x_0 and radius r, and let S denote the $(n-1)$ dimensional hypersurface area of this hypersphere. Evidently

$$V = \frac{2\pi^{n/2}}{n\Gamma\left(\frac{n}{2}\right)} r^n, \qquad S = \frac{2\pi^{n/2}}{\Gamma\left(\frac{n}{2}\right)} r^{n-1}. \quad (10)$$

Also $V/S = r/n$, and $dV = Sdr$.

Consider a particle P of mass m situated at a distance a from the center x_0 of the hypersphere. The hypersphere is supposed to be a homogeneous material hypersphere so that its density μ either per unit hypervolume or per unit hypersurface area is constant. Then its hypervolume mass is $M = \mu V$, and its hypersurface mass is $M = \mu S$, where V and S are given by (10).

If $n \geqq 3$, the potential function of the particle P due to the attraction of the material hypervolume of the hypersphere is

$$U(x) = \begin{cases} \dfrac{kmM}{(n-2)a^{n-2}} & \text{if } 0 < r < a < +\infty, \\ \dfrac{kmM}{r^n}\left[-\dfrac{a^2}{2} + \dfrac{nr^2}{2(n-2)}\right] & \text{if } 0 \leqq a \leqq r < +\infty. \end{cases} \quad (11)$$

If $n = 2$, the potential is

$$U(x) = \begin{cases} kmM \log \dfrac{1}{a} & \text{if } 0 < r < a < +\infty, \\ \dfrac{kmM}{r^2}\left(-\dfrac{a^2}{2} + \dfrac{r^2}{2} + r^2 \log \dfrac{1}{r}\right) & \text{if } 0 \leqq a \leqq r < +\infty. \end{cases} \quad (12)$$

The corresponding force of attraction for (11) and (12) is

$$X_i = \begin{cases} -\dfrac{kmM}{a^n}(x^i - x_0{}^i) & \text{if } 0 < r < a < +\infty, \\ -\dfrac{kmM}{r^n}(x^i - x_0{}^i) & \text{if } 0 \leqq a \leqq r < +\infty. \end{cases} \tag{13}$$

In the exterior of the hypersphere, the potential function U of (11) and (12) obeys the Laplace equation (9). In the interior, the potential function obeys the Poisson equation

$$\nabla^2 U = -km\mu \frac{2\pi^{n/2}}{\Gamma\left(\frac{n}{2}\right)} \qquad \text{if } 0 \leqq a < r < +\infty. \tag{14}$$

If $n \geqq 3$, the potential function of the particle P due to the attraction of the material hypersurface of the hypersphere is

$$U(x) = \begin{cases} \dfrac{kmM}{(n-2)a^{n-2}} & \text{if } 0 < r < a < +\infty, \\ \dfrac{kmM}{(n-2)r^{n-2}} & \text{if } 0 \leqq a \leqq r < +\infty. \end{cases} \tag{15}$$

If $n = 2$, then

$$U(x) = \begin{cases} kmM \log \dfrac{1}{a} & \text{if } 0 < r < a < +\infty, \\ kmM \log \dfrac{1}{r} & \text{if } 0 \leqq a \leqq r < +\infty. \end{cases} \tag{16}$$

The corresponding force of attraction for (15) and (16) is

$$X_i = \begin{cases} \dfrac{-kmM}{a^n}(x^i - x_0{}^i) & \text{if } 0 < r < a < +\infty, \\ \dfrac{-kmM}{2a^n}(x^i - x_0{}^i) & \text{if } a = r, \\ 0 & \text{if } 0 \leqq a < r < +\infty. \end{cases} \tag{17}$$

The potential function U of (15) and (16) obeys the Laplace equation (9) in the exterior and interior of the hypersphere.

For the classical three-dimensional case, see O. D. Kellogg, *Foundations of Potential Theory*, Frederick Ungar Publishing Company, New York.

INFORMATION TO CONTRIBUTORS

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